Critical Reviews in Biochemistry and Molecular Biology, 40:155–179, 2005

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230590954153

Taylor & Francis
Taylor & Francis Group

Perspective on Mutagenesis and Repair: The Standard Model and Alternate Modes of Mutagenesis

Jeffrey H. Miller
Department of Microbiology, Immunology, and Molecular Genetics, and The Molecular Biology Institute, University of California, Los Angeles, CA, USA

ABSTRACT The basic ideas of replication, mutagenesis, and repair have outlined a picture of how point mutations occur that has provided a valuable framework for theory and experiment, much as the Standard Model of particle physics has done for our concept of fundamental particles. However, alternative modes of mutagenesis are being defined that are changing our perspective of the "Standard Model" of mutagenesis, requiring an expanded model. The genome is now envisioned as being in dynamic equilibrium between a multitude of forces for mutational change and forces that counteract such change. By maintaining a delicate balance between these forces, cells avoid unwanted or excessive mutations. Yet, cells allow mutagenesis to occur under certain conditions. We can define an emerging paradigm. Namely, mechanisms exist that can direct point mutations to specific designated genes or regions of genes. In some cases, this is achieved by specific enzymes, and in other cases high mutability is programmed into the sequence of certain genes to help generate diversity. In yet additional cases, general mutability is increased under stress, and selective forces allow the recovery of favorable mutants.

KEYWORDS mutagenesis, repair, standard model, alternate pathways, directed mutations

INTRODUCTION

In reading Gordon Kane's recent Scientific American article "Physics Beyond the Standard Model" (Kane, 2003), I was struck by some of the parallels between the development of models for particle physics, and those of molecular biology, and in particular models of mutagenesis. Just as the "Standard Model" of particle physics has stood for 30 years, but is now on the verge of requiring significant extensions, the basic concept of mutagenesis is also on the threshold of significant change. Although no one has actually attached a label to the concordance of ideas on mutagenesis in the same way that Francis Crick did with the "Central Dogma,"–which states that information flows from nucleic acid into protein and never in reverse (see Judson, 1979)–I would offer that there has been a basic or "Standard Model" of mutagenesis and repair that emerged out of early work and that took its present form after the elucidation of the structure of DNA and work through the 1970s and the 1980s. This model has stimulated

Editor: Michael M. Cox

Address correspondence to Jeffrey H. Miller, Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095. E-mail: jhmiller@mbi.ucla.edu

a large amount of research aimed at filling in its details and at applying it to human disease,especially to cancer. The Standard Model is not a mere formulation, but rather the result of several decades of experimentation. It reduces a complex series of events into a set of unified and simplified processes. Although first established for bacteria and bacteriophage, its principles also apply to higher cells, from yeast to humans.

Now, however, alternative mechanisms of mutagenesis are emerging that help define a new paradigm. Mutations are not always an accident of unrepaired replication errors or DNA damage, but sometimes result from pathways that are outside the Standard Model. Some of these pathways are even programmed to create mutations. As the study of mutagenesis expands to include more of the enormous diversity of organisms, we can only expect to find additional examples of pathways that deviate from the Standard Model. Let us review the Standard Model and examine some of the alternate pathways.

THE STANDARD MODEL OF MUTAGENESIS AND REPAIR

In the Standard Model (Figures 1 to 4), DNA is normally replicated by DNA polymerases (Figure 1) that use recognition of specific base pairs together with Watson-Crick base-pairing specificity to effect a certain level of fidelity (reviewed in Kornberg & Baker, 1992; Friedberg *et al.*, 1995). This fidelity is enhanced both by editing subunits of the polymerase itself (Scheuermann *et al.*, 1983; Scheuermann & Echols, 1984; Echols & Goodman, 1991), and by enzymes that recognize and correct replication errors (mismatch repair; Figure 2; reviewed in Modrich, 1991; Modrich & Lahue, 1996; Kolodner, 1996). Mutations occur as a result of replication errors that escape repair, as a consequence of spontaneous DNA damage, or as a result of the action of mutagens. Some mutagens are base analogs, whereas others cause alterations or damage to base pairs that lead to mispairing and thus to higher rates of errors during replication, as described below. (Here we are concerned with point mutations, base substitutions, and small additions or deletions. Larger rearrangements, such as large deletions, insertions, or inversions, including those generated by transposable elements, are not described by the Standard Model). DNA damage can occur by agents generated during cellular metabolism (endogenous), or by external agents (exogenous), and is subject to repair by a wide variety of repair systems. Some categories of mutagens that fit the Standard Model and some repair systems are summarized below.

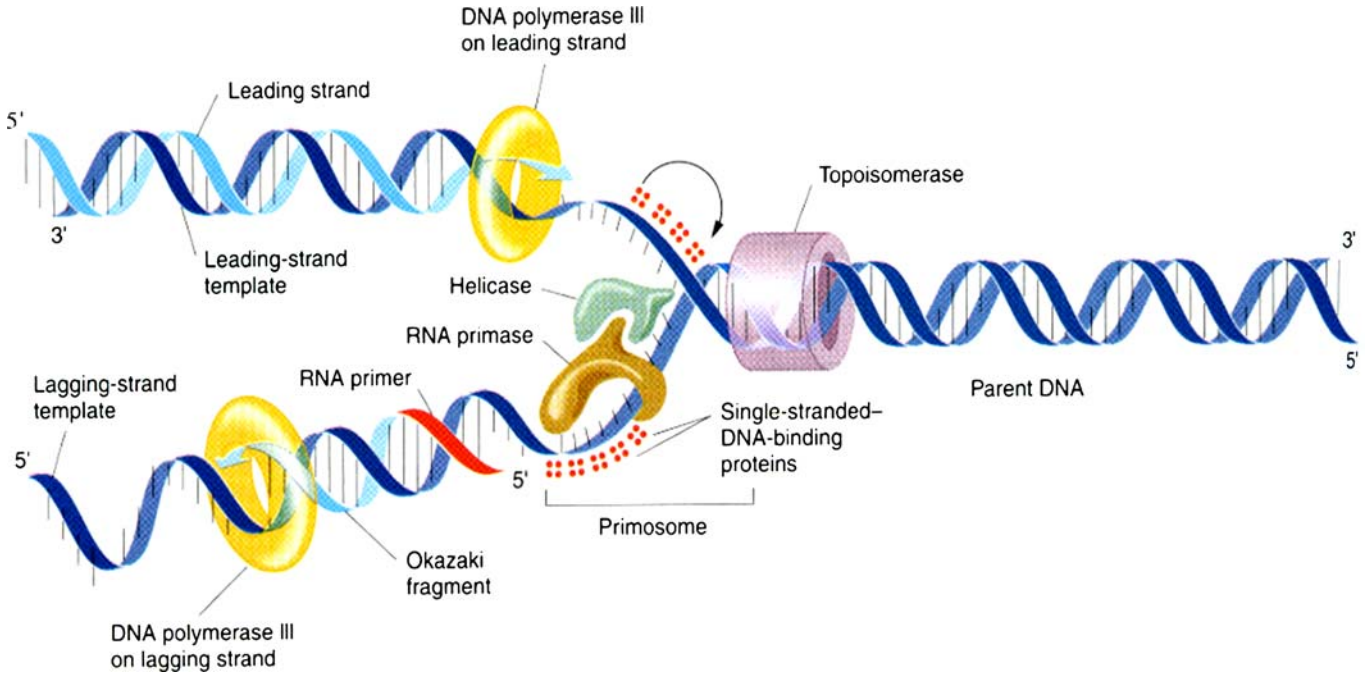

FIGURE 1 **DNA replication in *E. coli.* A schematic view of many of the essential elements of DNA replication at the replication fork. (From Griffiths *et al.*, 1999).**

Base Analog Induced Replication Errors

One class of mutagens studied during the initial work on mutagenesis in phage and bacteria are termed base analogs, since they can be incorporated into DNA but have more ambiguous pairing rules than do normal bases (for more detailed descriptions, see Friedberg *et al.*, 1995). 2-aminopurine (2AP) is an example of this type of mutagen. 2AP is an analog of adenine (A), but in addition to pairing with T, both its enol and ionic tautomers can pair well with C. These tautomers occur more frequently than those of the normal Watson-Crick bases. Thus, 2AP

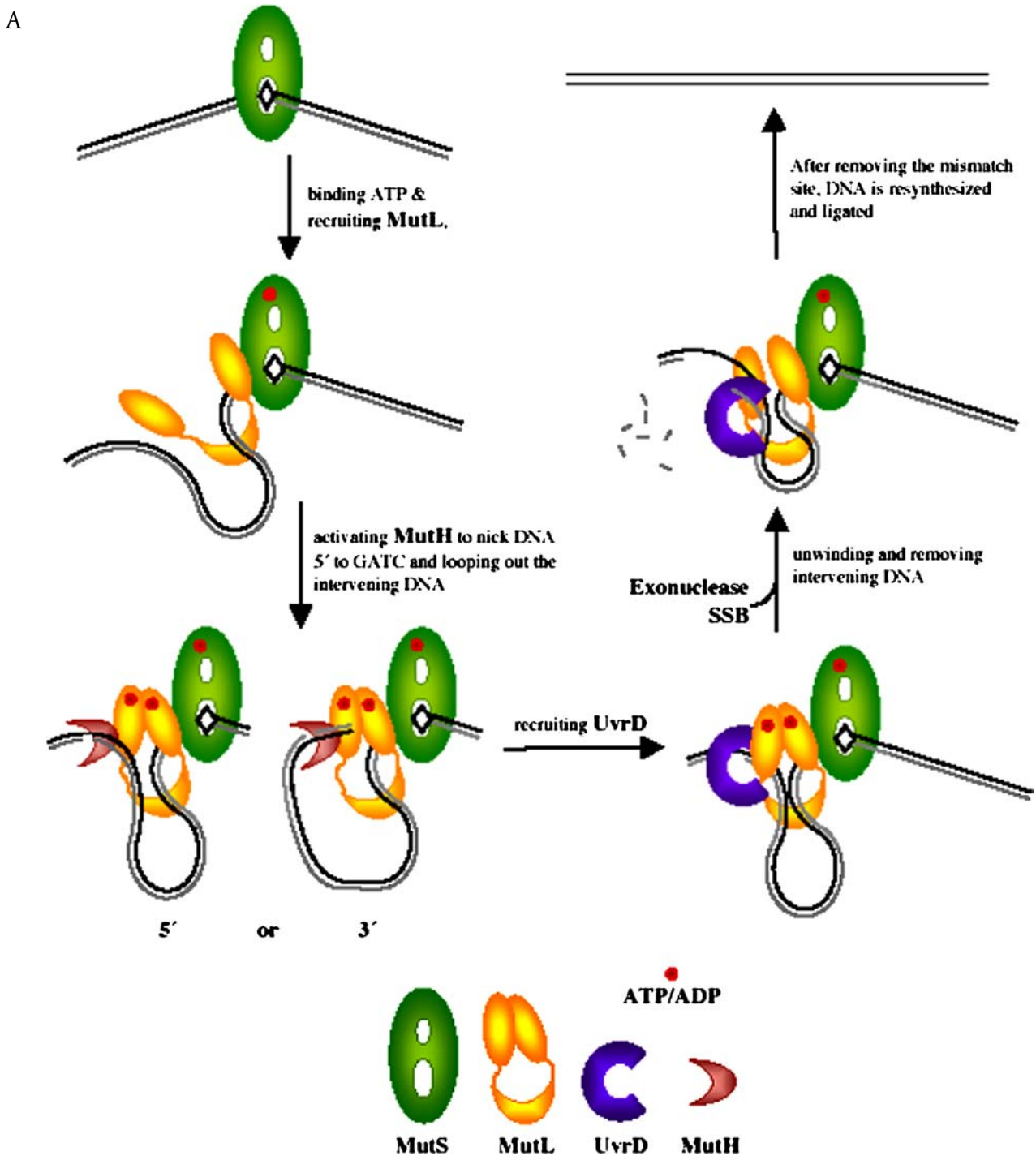

FIGURE 2 A) A model of MutL-mediated mismatch repair in *E. coli.* (From Guarné *et al.,* 2004). B) Functional networks in DNA damage recognition by MMR proteins. (From Bellacosa, 2001). *(Continued)*

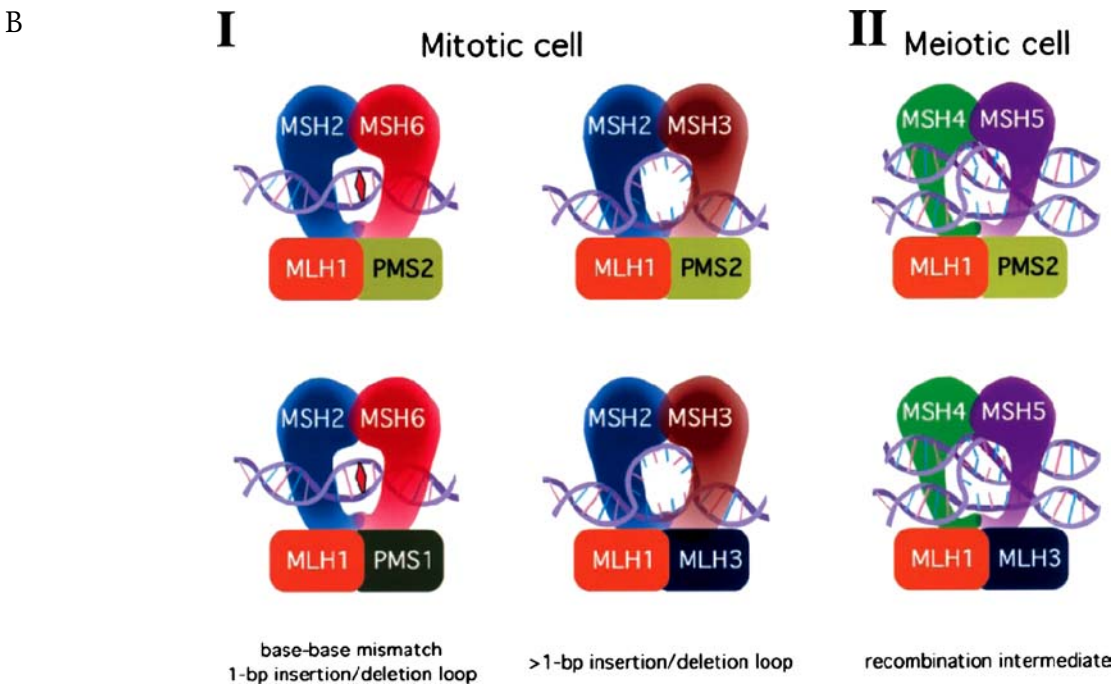

FIGURE 2 (Continued).

increases mutations from G:C → A:T and vice versa. (Figure 3).

Damage to DNA

There are many types of damage to DNA that can be caused by exposure to different agents. One example involves alkylating agents, such as ethyl methanesulfonate, a mutagen that alkylates the 0^6 position of guanine, among other positions, converting the guanine into a base that now pairs with T (Figure 4a). Alkylating agents can be powerful mutagens. Oxidative damage can occur as a result of exposure to ionizing radiation, or oxidizing agents such as hydrogen peroxide, or those generated during metabolism. 8-oxodGuanine is a frequent product of oxidative damage (Figure 4b). The substitution at the 8-position of guanine pushes the G into the *syn* conformation, thus allowing mispairing with adenine and resulting in G → T transversions. Another example involves UV irradiation-induced photoproducts, including two different types of pyrimidine-pyrimidine dimers (Figure 4c). This latter type of damage, as well as AP (apurinic or apyrimidinic) sites resulting from cleavage of the N-glycosydic bonds, involves loss of pairing specificity, so that replication across from such lesions usually leads to mutations (see also below). Most of the types of DNA damage that lead to mutagenesis are described in detail in the volume by Friedberg and coauthors (1995) (see also reviews by Barnes & Lindahl, 2004; Friedberg *et al.*, 2004).

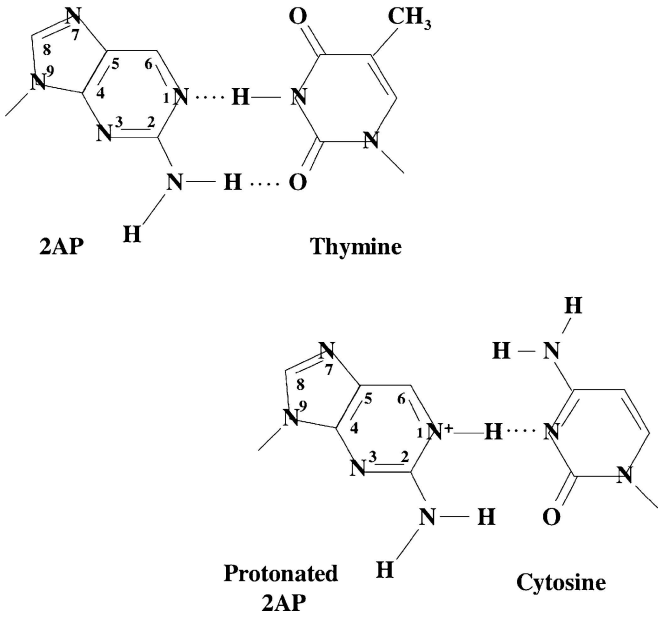

FIGURE 3 2-aminopurine (2AP) paired with thymine, and a protonated form of 2AP paired with cytosine.

DNA Repair Systems

In addition to the polymerase editing function and the mismatch repair system that operate on replication errors, a myriad of DNA repair systems has been characterized (see Friedberg *et al.*, 1995; Lindahl & Barnes, 2000; Wood *et al.*, 2001; Lindahl, 2001; Friedberg, 2003; Barnes & Lindahl, 2004). Table 1 summarizes many

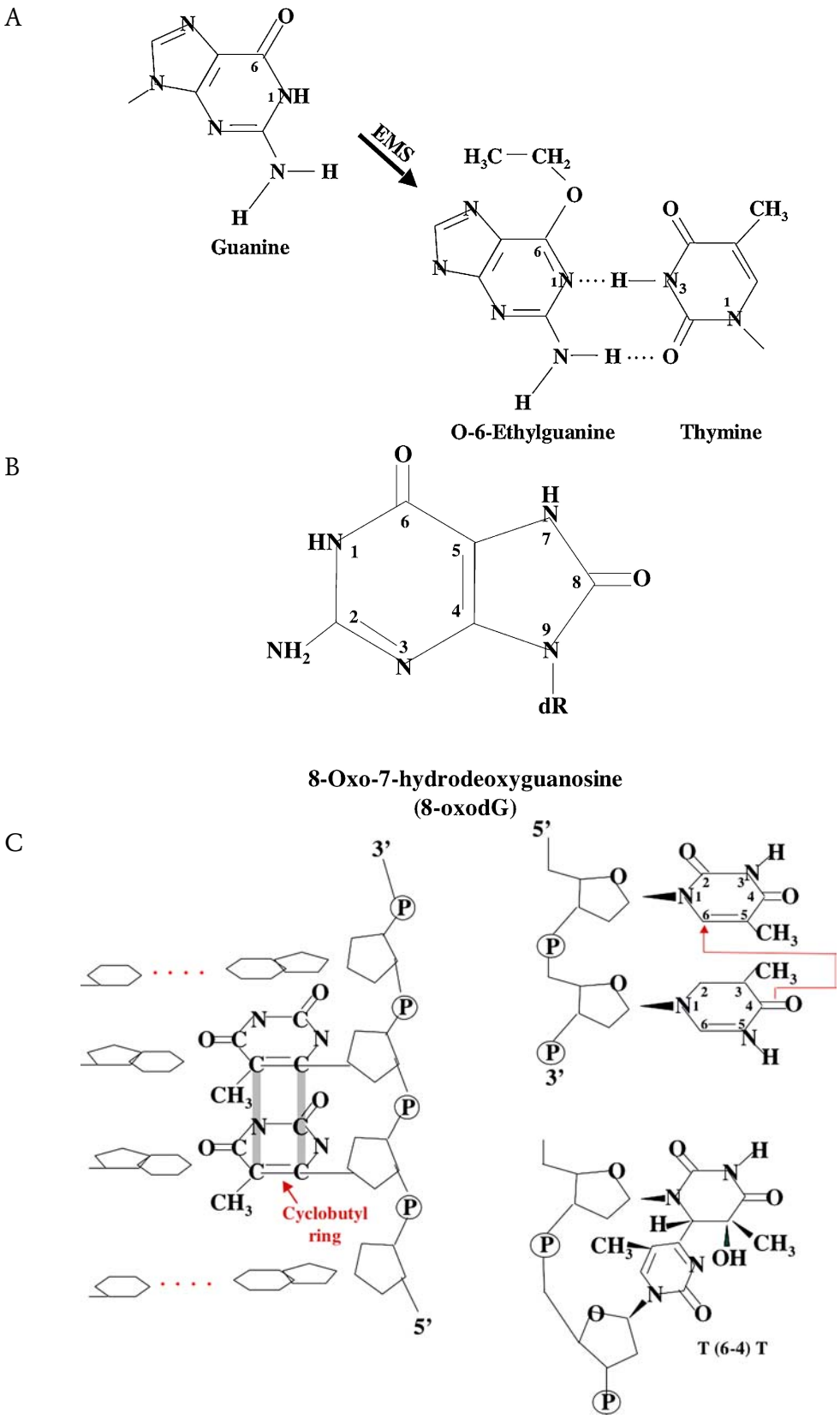

FIGURE 4 A) EMS alkylates the O-6 position of guanine, generating mispairs with thymine. B) Oxidation of the C-8 position of guanine. C) Structures of a cyclobutane pyrimidine dimer (left) and of the 6-4 photoproduct (right). (From Friedberg *et al.*, 1995).

TABLE 1 **Types of repair systems characterized in *Escherichia coli***

General mode of operation	Example	Type of lesion repaired	Mechanism
Detoxification	Superoxide dismutase	Prevents formation of oxidative lesion	Converts peroxides into hydrogen peroxide, which is neutralized by catalase
Direct removal of lesions	Alkyltransferases	O-6-alkylguanine	Transfers alkyl group from O-6-alkylguanine to cysteine residue on transferase
	Photolyase	6-4 photoproduct	Breaks 6-4 bond and restores bases to normal
	Photolyase	UV photodimers	Splits dimers in the presence of white light
	Oxidative demethylation	1-methyadenine, 3-methylcytosine	AlkB catalyzes the oxidation of the methyl groups, requiring O_2, α-ketoglutarate, and Fe(II), releasing it as formaldehyde
General excision	*uvrABC*-encoded exonuclease system	Lesions causing distortions in double helix, such as UV photoproducts and bulky chemical additions	Makes endonucleolytic cut on either side of lesion; resulting gap is repaired by DNA polymerase I and DNA ligase
Specific excision	AP endonucleases	AP sites	Makes endonucleolytic cut; exonuclease creates gap, which is repaired by DNA polymerase I and DNA ligase
	DNA glycosylases	Deaminated bases (uracil, hypoxanthine), certain methylated bases, ring-III opened purines, oxydatively damaged bases; and certain other modified bases	Removes base, creating AP site, which is repaired by AP endonucleases
	GO system	8-oxodG	The MutM glycosylase removes 8-oxodG from DNA; the MutY glycosylase removes the A from 8-oxodG-A mispairs, leading to re-creation of an 8-oxodG-C pair, and the MutM glycosylase then removes the 8-oxodG
Postreplication	Mismatch repair system	Replication errors resulting in base-pair mismatches	Recognizes newly synthesized strand by detecting nonmethylated adenine residues in 5′-GATC-3′ sequences; then excises bases from the new strand when a mismatch is detected
	Recombinational repair	Lesions that block replication and result in single-stranded gaps	Recombinational exchange
	SOS system	Lesions that block replication	Activates synthesis of DNA polymerases that allow replication bypass of blocking lesion, resulting in frequent mutations across from lesion, and also mutations across from normal bases

For further details see reviews by Friedberg *et al.*, 1995; Friedberg, 2003; Barnes and Lindahl, 2004; Friedberg *et al.*, 2004; and also Trewick *et al.*, 2002; and Falnes *et al.*, 2002. Table modified from Griffiths *et al.*, 1999.

of the known repair systems in bacteria. Their normal functioning results in spontaneous base substitution rates averaging on the order of 10^{-10} per base pair per replication,in bacteria such as *E. coli*, although this will vary considerably at individual sites (e.g. Garibyan *et al.*, 2003). Base substitution rates also vary from one organism to another (*e.g.*, Drake, 1991). However, the total genomic substitution rates vary within a much narrower

range, and are in fact relatively constant (Drake, 1991; Drake *et al.*, 1998).

ALTERNATE MODES OF MUTAGENESIS—EXTENSIONS TO THE STANDARD MODEL

More than 10 pathways of mutagenesis have been defined or outlined that are distinct enough from the basic mutagenesis paradigm to let us categorize them as alternate modes of mutagenesis not covered by the Standard Model. Although the original studies of some of these pathways go back several decades, much of this work has been completed relatively recently.

The Induction of New Polymerases for SOS Bypass

The SOS system was first described following studies of UV irradiation (Radman, 1974, 1975; Witkin, 1974, 1976), and a large body of literature is devoted to this system, which involves the expression of a set of genes in response to lesions that block DNA replication (reviewed in Walker, 1987; Friedberg *et al.*, 1995). In addition certain UV photoproducts, AP sites and some bulky adducts also prevent normal base pairing, triggering the replication machinery to stop at that point (see Courcelle *et al.*, 2001, and references therein). Subsequent restarting past the blocking lesions leaves gaps that need to be filled. When these gaps persist, a set of proteins is induced that, among other things, allows replication past the blocking lesion. Recently, it was discovered that special DNA polymerases, such as Pol IV and Pol V in *Escherichia coli*, are induced that can replicate past these blocking lesions (Reuven *et al.*, 1999; Tang *et al.*, 1999; Wagner *et al.*, 1999). These polymerases are part of the "Y super family" of error prone polymerases that occur in bacteria, yeast, and humans (reviewed in Friedberg *et al.*, 2002; Ohmori *et al.*, 2001; Sutton & Walker, 2001). Figure 5 diagrams their mode of action in *E. coli*, yeast, and humans. The mutations made across from the blocking lesions fall within the confines of the Standard Model. However, these polymerases have a less stringent fidelity, and as a result make errors across from normal bases at a higher frequency than do the normal replication and repair polymerases. Therefore, mutations are made at sites that are distant from the actual lesions, as shown schematically in Figure 6. These latter mutations, originally termed "untargeted mutations" (Witkin & Wermundsen, 1978), fall outside the Standard Model. The level of these mutations is reduced by the mismatch repair system (Caillet-Fauguet & Maenhaut-Michel, 1988; Fijalkowska *et al.*, 1997), and is usually low when compared with the level of mutagenesis resulting from replication across from the noncoding lesions. However, there are situations when this level can be elevated, such as by overexpressing Pol IV encoded on a plasmid (Kim *et al.*, 1997; Yang *et al.*, 2004), and there are potential mutagens that could operate solely indirectly by inducing this system. Moreover, other stress responses also involve overexpression of Pol IV, such as the stationary phase stress response induced by the RpoS sigma factor in *E. coli* (Layton & Foster, 2003; Lombardo *et al.*, 2004). Mutants have been described that have this system permanently turned on (Witkin, 1974), and these have an elevated level of mutagenesis (Witkin, 1974; Miller & Low, 1984) resulting from these "error-prone" polymerases. It has been suggested by a number of authors that activating the SOS system is a mechanism designed for generating variants during times of stress (reviewed in Radman *et al.*, 2000), although it may be that the additional mutagenesis caused by activating the Y family polymerases is simply an unavoidable consequence of bypassing noncoding lesions.

Adaptive Mutagenesis

Mutations that appear in non-growing or very slowly growing cells and only in the presence of selective agents, a phenomenon that was first recognized as such by Cairns and coworkers (1988), have been termed "adaptive mutations." A large body of work by several laboratories (see recent reviews by Foster, 2004a, b, c; Rosenberg & Hastings, 2004a, b, c; and Roth & Andersson 2004a, b, c) has sought to define the pathway by which these mutations occur. The mutations preferentially occur on the F′ lac episome (plasmid) used in many experiments. There are disagreements over the mechanism, with some groups favoring the induction of increased mutagenesis in a segment of the population (see Foster, 2004a; Rosenberg & Hastings, 2004a), perhaps involving the RpoS sigma-dependent stress response (Layton & Foster, 2003; Lombardi *et al.*, 2004; see above section), with mechanisms based on double strand break repair accounting for many of the remaining mutations. On the other hand, Roth and coworkers (2004c) maintain that all the mutations are the result

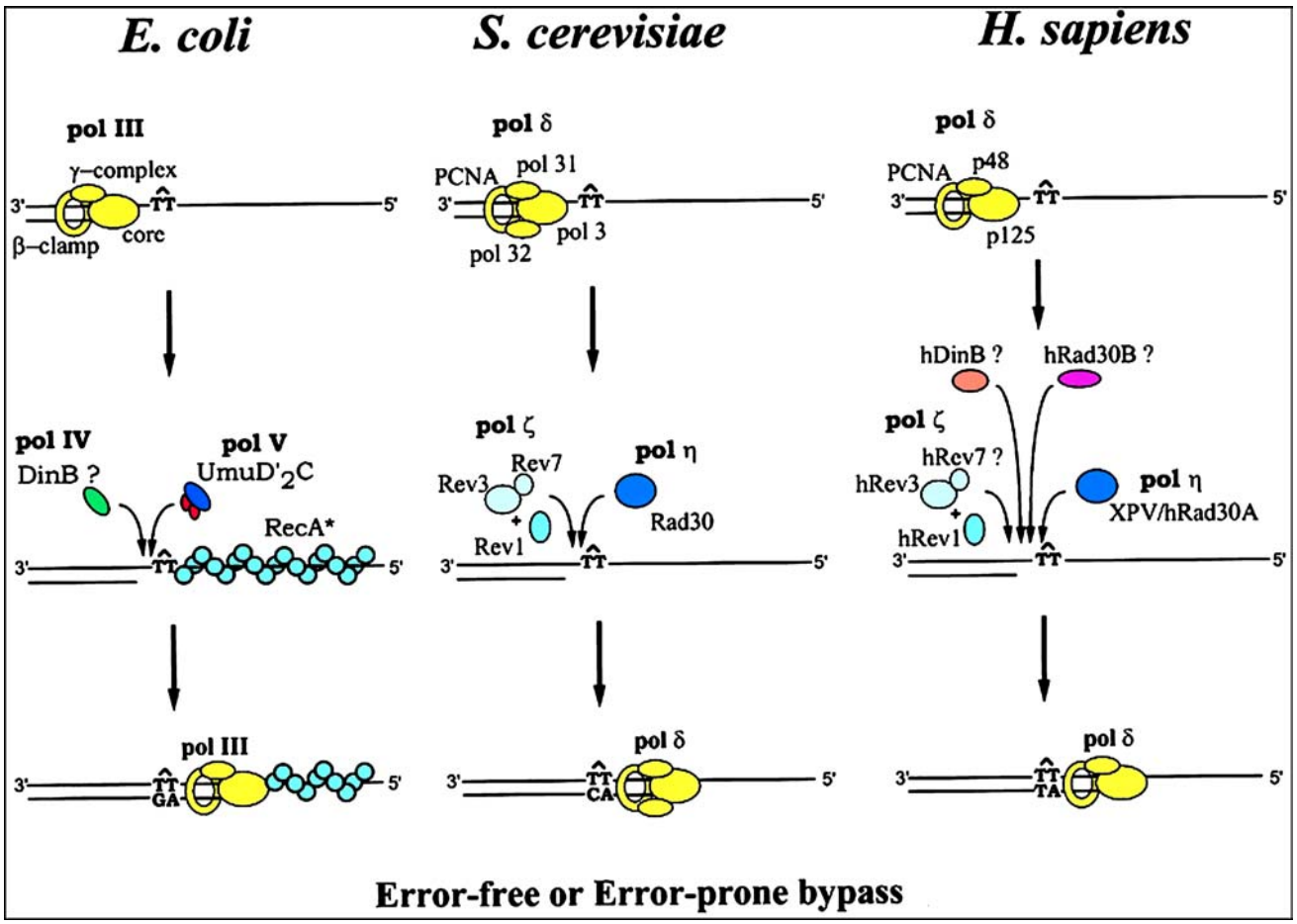

FIGURE 5 Conserved mechanism of translesion DNA synthesis. Here the replicating polymerase is displaced by the bypass polymerases that carryout translesion synthesis, and then are subsequently replaced by the original replicating polymerase (After Woodgate, 1999).

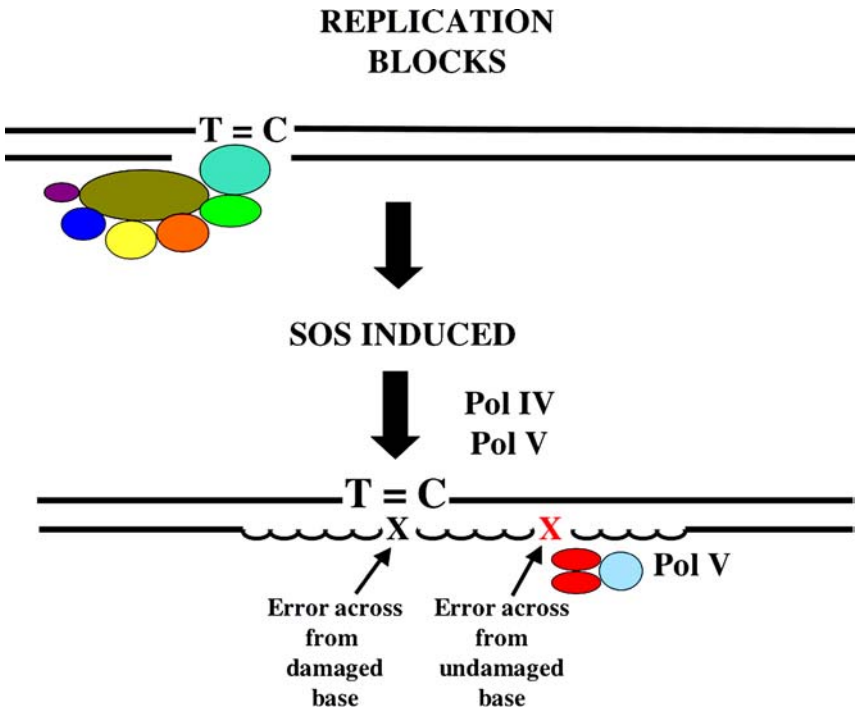

FIGURE 6 Mutations generated by replication errors from bypass polymerases. DNA polymerase is shown in a highly schematic form.

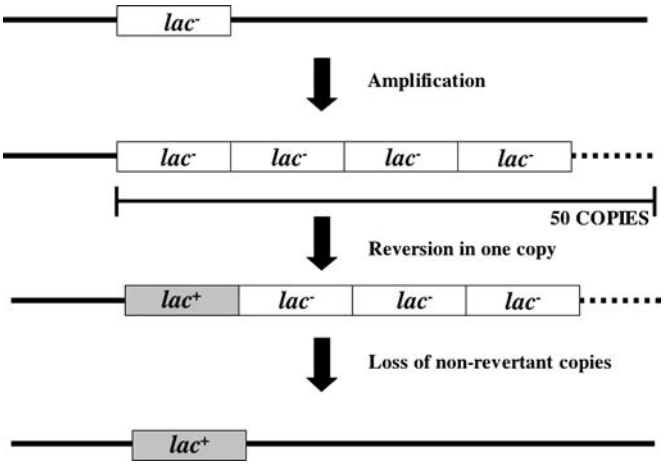

FIGURE 7 **Model for reversion by first amplifying copies to increase the chance that one copy undergoes mutation, and then losing the other copies.**

of amplification of the *lac* region of F, generating 50 to 100 copies that provide an increased chance for a revertant to occur (Figure 7). Once this happens, the single copy of the reverted gene is maintained, but the F plasmid rapidly loses the now unneeded extra copies. Amplification of genes in bacteria (Edlund & Normark, 1981), and of the *lac* region (Horiuchi *et al.*, 1963; Tlsty *et al.*, 1984; Whoriskey *et al.*, 1987), have been described, and amplification-based models have been considered for adaptive mutation (Foster & Cairns, 1992; Andersson *et al.*, 1998; Hastings *et al.*, 2000; Hendrickson *et al.*, 2002). The transient increase in copy number to increase the chance of getting a revertant is a novel route to increased mutagenesis, particularly when the revertant no longer carries the amplifcation. Whether this is the sole or virtually sole mechanism for adaptive mutagenesis, as Roth's group argues (Roth & Andersson, 2004a, b, c) or only one of several ways that result in these mutations (Foster 2004a, b, c; Rosenberg & Hastings, 2004a, b, c), such transient amplification clearly represents an alternate mode of mutagenesis. Whether this is essentially restricted to certain specific F′ derivatives or is more widespread cannot be answered at this point, since our view of mutagenesis is based on such a relatively small segment of the microbial realm, not to mention the entire living world. It should be noted that, as a phenomenon, gene amplification has been well documented in eukaryotes (*e.g.*, Schimke, 1982).

Cairns and coworkers (1988) originally suggested that the mutations in *lac* might represent an example of directed mutagenesis (see also Shapiro, 1984; Hall, 1997; and discussion in Cairns, 1998). Although not strictly true in this case, according to the current models, this idea is in fact born out in several other systems reviewed below.

Mistranslation Mutagenesis

In our laboratory, we detected two mutators, *mutA* and *mutC* (Michaels *et al.*, 1990) that result from mutations in the genes for different copies of a glycine tRNA (Slupska *et al.*, 1996). The mutations affect the anticodon, so the tRNAs now read aspartic acid codons and insert glycine at these codons (Slupska *et al.*, 1996), although at a low percentage (1%). How could 1% mistranslation produce a mutator effect? In other words, how could smaller amounts of an altered protein cause such an effect in the presence of large amounts of the normal counterpart? A good candidate is DNA polymerase, or a component of DNA polymerase, such as the proofreading subunit ε. Certain mutations in the *dnaQ* gene encoding ε create a strong mutator phenotype, *mutD* (Degnen & Cox, 1974; Cox & Horner, 1982; Echols *et al.*, 1983; Maruyama *et al.*, 1983), and these mutations are dominant to wild-type. As it turns out, 1% of the level of mutagenesis resulting from *mutD* would account for the level of mutagenesis seen in strains with *mutA* or *mutC*. These considerations resulted in the idea that in *mutA* or *mutC* strains mutator polymerases, with an altered ε subunit, are continually and transiently created in a subset of the population by mistranslation, in this case the specific mistranslation of GLY for ASP (Slupska *et al.*, 1996) (Figure 8). This notion is supported up by the demonstration that substitutions of glycine for aspartic acid at two different positions in the *dnaQ*-encoded epsilon are strong mutators, similar to the *mutD* mutator reported for other alterations in the *dnaQ* gene (Slupska et al., 1998). Moreover, specific mistranslation of glycine for histidine produces a similar effect (Slupska *et al.*, 1998). Humayan and coworkers (Humayun, 1998; Murphy *et al.*, 1997; Balashov *et al.*, 2004, and references therein) have suggested a different mechanism emanating from mistranslation in general that induces a mutagenic effect, although again involving polymerase, and probably also the epsilon subunit (Balashov *et al.*, 2004). Independent of the details of the mechanism, mistranslation clearly offers another alternative mode of mutagenesis not covered by the Standard Model. It should be noted here that one of the above models of mutagenesis resulting

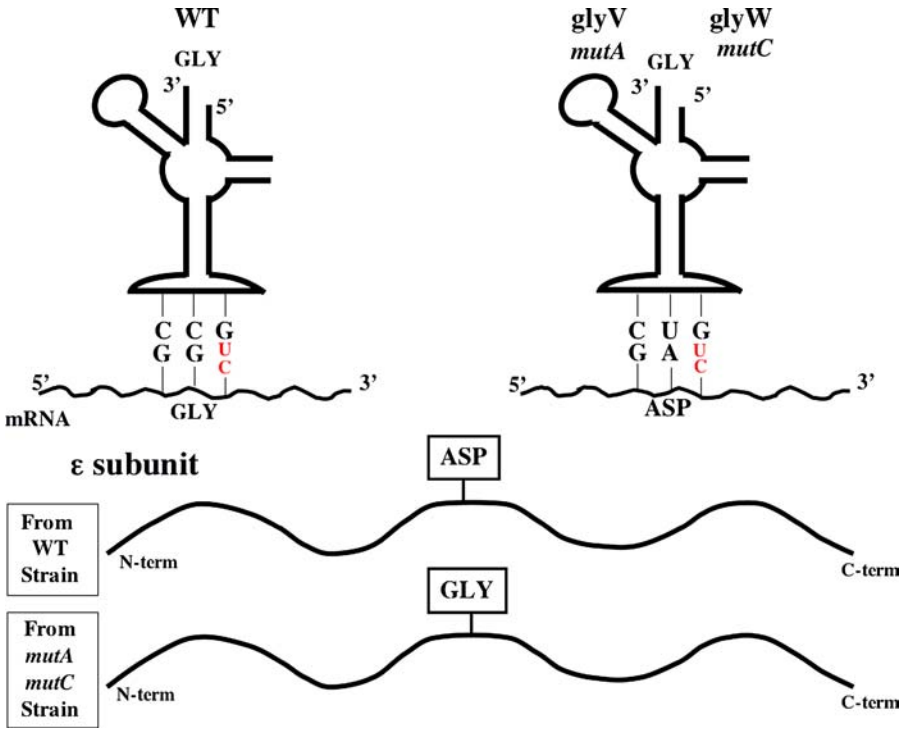

FIGURE 8 **Model for action of mutator tRNAs. Here, the insertion of glycine at aspartic acid codons at low frequency generates a population of altered DNA polymerase proofreading subunits (ε). (From Slupska *et al.*, 1996.)**

from mistranslation represents an example of transient mutators postulated by Ninio (1991), who suggested that partitioning of key components required for translation and transcription, and also editing and mismatch repair, might generate cells that make more uncorrected errors for one generation.

Quasi-Palindrome Templated Mutations

DNA has the ability to partially denature for short stretches and to form secondary structures. Cruciform structures can result from quasipalindromes that fold back, creating imperfect duplexes, as shown in Figure 9. If the duplex segment is pronounced enough, the mispairs can be resolved in one direction or the other, resulting in mutations that are often multiple mutations. Ripley and coworkers (Ripley, 1982; de Boer & Ripley, 1984) first proposed and then demonstrated this "templating" of point mutations in frameshifts in bacteriophage T4 (reviewed in Lovett, 2004.) Susan Lovett and coworkers (Viswanathan *et al.*, 2000) showed that, in the *thyA* gene of *E. coli*, a templated mutation (Figure 10) is the most frequent spontaneous mutation, and one of the most frequent mutations studied so far in *E. coli*. In the detailed model depicted in Figure 10, exonucleolytic degradation and resynthesis on the lagging strand resolves the mispairs (Viswanathan *et al.*, 2000). This pathway of mutagenesis (see also Yoshiyama & Maki, 2003), which one might envision as fooling repair synthesis into making mutations, is a clear departure from the Standard Model, and requires an extension of the model.

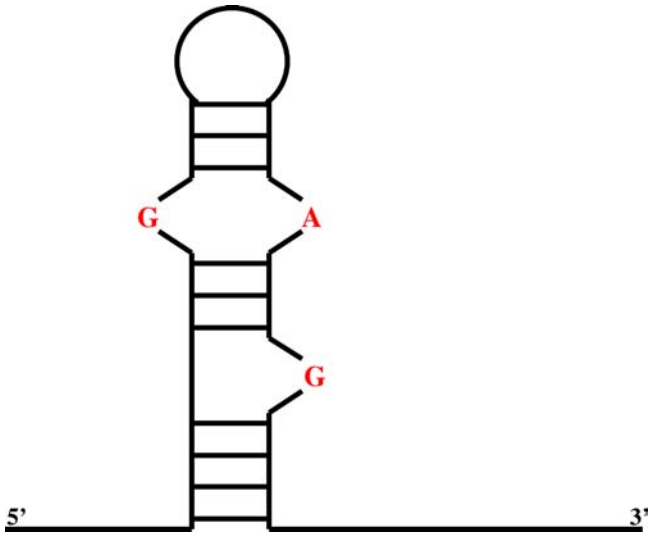

FIGURE 9 **An example of a cruciform structure that could form from a quasipalindrome.**

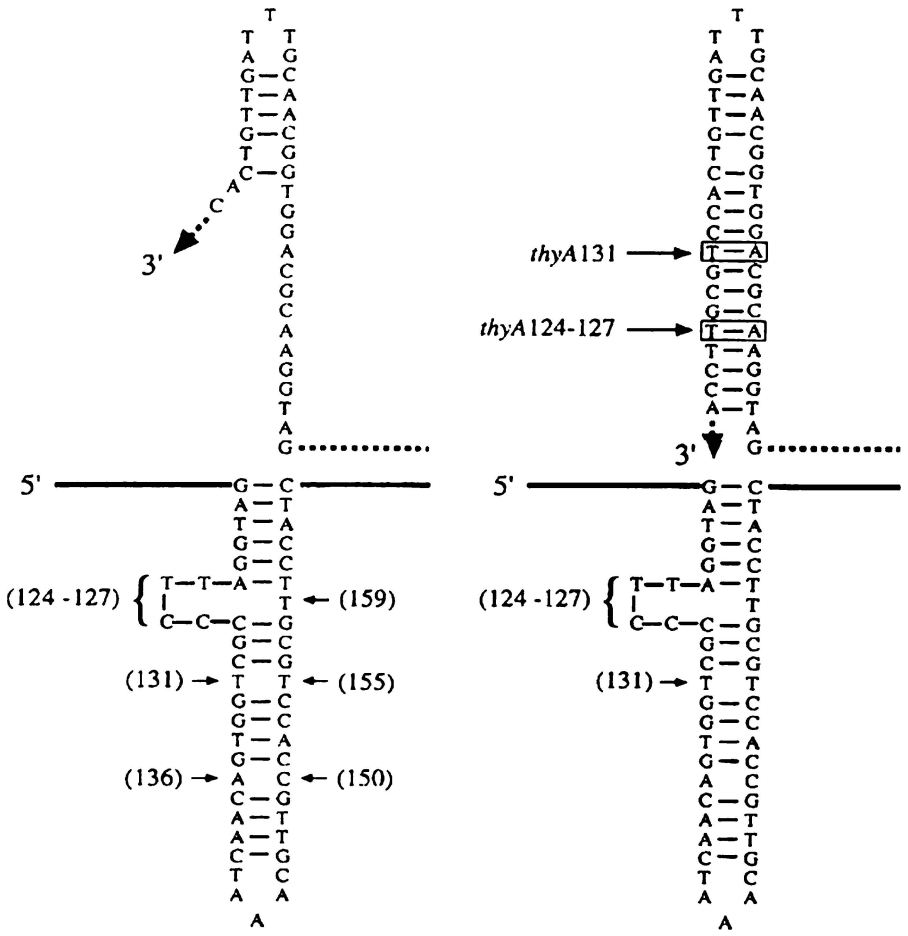

FIGURE 10 **Hairpin-templated mutagenesis mechanism for a hotspot in thyA. During lagging strand replication, the nascent strand undergoes intramolecular pairing, forming cruciform structures (Bottom left, right. Unpaired nucleotides at the 3′ end of the nascent strand are vulnerable to degradation by ExoI or ExoVII (top left). DNA replication converts the structure into a more fully paired hairpin, introducing new mutagenic changes (thyA131 and thyA1240127) into previously unpaired sites. (From Viswanathan *et al.*, 2000).**

Extensive Repeat Templated Mutations

Accelerated Triplet Repeat Expansion

As many as 15 neurodegenerative diseases in humans result from the expansion of a triplet that is present as a tandem array in normal individuals, but that expands to very large numbers in afflicted individuals after an intermediate stage with modestly increased triplet numbers (reviewed in Wells, 1996; Gordenin *et al.*, 1997; Richard *et al.*, 1999; Mirkin & Smirnova, 2002). An example is Fragile X syndrome, resulting from CGG triplet expansion (Figure 11). Normal individuals have 6 to 54 copies of a CGG repeat in a protein involved in neural development. This expands to 54 to 200 copies in "pre-mutational" individuals, and from 200 to 1,300 copies in people with full symptoms. Other examples are Kennedy and Huntington disease, both resulting from the expansion of the CAG triplet, and myotonic dystrophy, resulting from the expansion of the CTG triplet. Although increases and decreases in tandemly repeating units can occur frequently as replication errors in the Standard Model (*e.g.*, Streisinger *et al.*, 1966), and were recognized as being responsible for certain mutational hotspots (*e.g.*, Farabaugh *et al.*, 1978; reviewed in Lovett, 2000), the accelerated run-up of repeat numbers seen here involves additional mechanisms that qualify as alternate modes of mutagenesis. The favored model involves secondary structures folding back on themselves and driving increased replication errors, as shown in Figure 12. For more detailed models see Wells (1996) and Gordenin *et al.* (1997).

Programmed High Mutation Rate at "Contingency" Loci

One mechanism for increasing genetic diversity involves loci that have high rates of mutation because they contain contain significant repeat-tract sequences

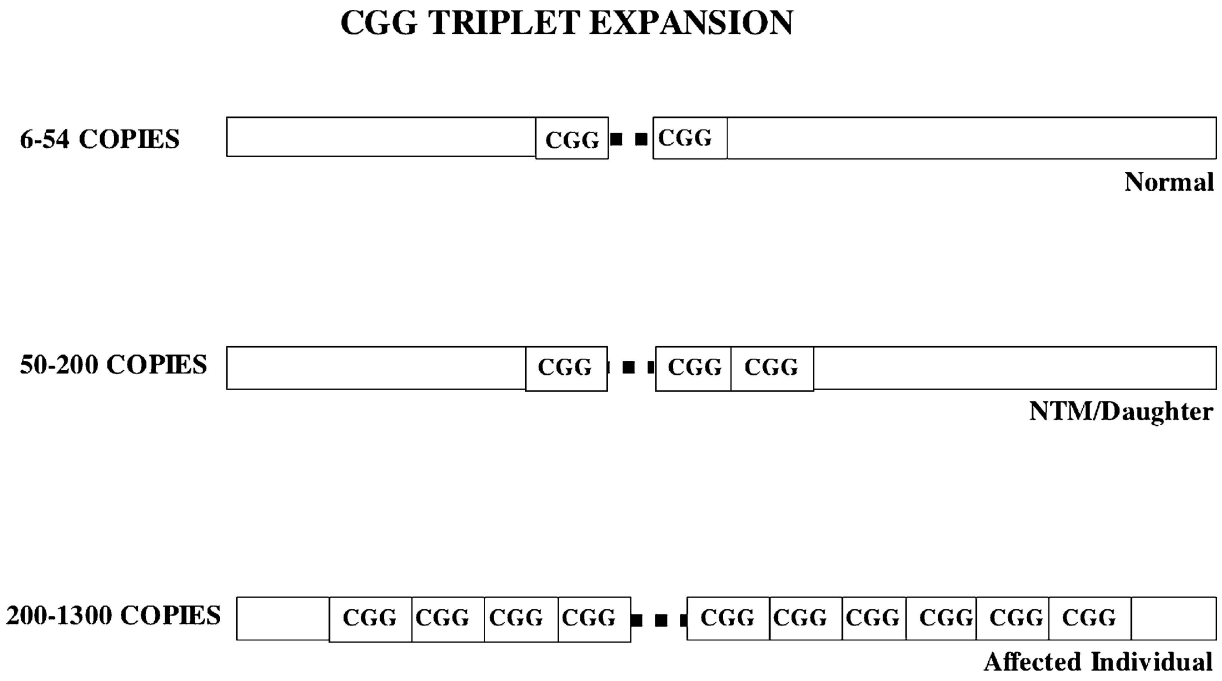

FIGURE 11 Triplet repeat expansion leading to Fragile X syndrome.

that are not multiples of three. These so-called "contingency" loci (Moxon, 1994; Bayliss *et al.*, 2001; see reviews by van Belkum *et al.*, 1999; Bayliss *et al.*, 2004a.) encode proteins such as surface antigens that give an organism a selective advantage if variation occurs frequently. Two well studied examples are *Haemophilus influenzae* and *Neisseria meningitides* (Bayliss *et al.*, 2001; 2004b; Martin *et al.*, 2004). Phase variation, the reversible switching of surface antigens, is achieved by frameshifting at sequence repeats in promoters or coding sequences. As many as 47 genes in *N. meningitides* are regulated by this mechanism (Martin *et al.*, 2004). For example, the *opa* genes contain repeats of a 5-bp sequence in the coding region, and a set of loci in *H. influenzae* contain repeats of a 4-bp sequence. Also, the divergent promoter for the *hifA* and *hifB,C,D,E* operons that are involved in pilus formation contain a–TA-repeat tract between the –35 and –10 regions. It is the programmed accelerated mutation rate at designated sites that qualifies contingency loci as a special mechanism.

Activation-Induced Cytidine Deaminase (AID)

In vertebrates, one of the mechanisms for diversification of immunoglobulin variable genes is somatic

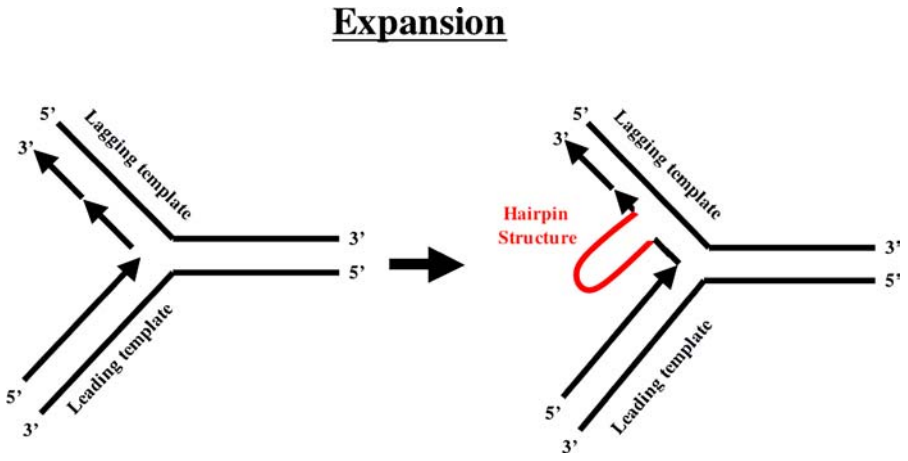

FIGURE 12 Model for accelerated triplet repeat expansion. Formation of a hairpin structure by an Okazaki fragment allows one region of the template to be replicated twice, leading to expansion (From Gordenin *et al.*, 1997, as adapted from Kang *et al.*, 1995).

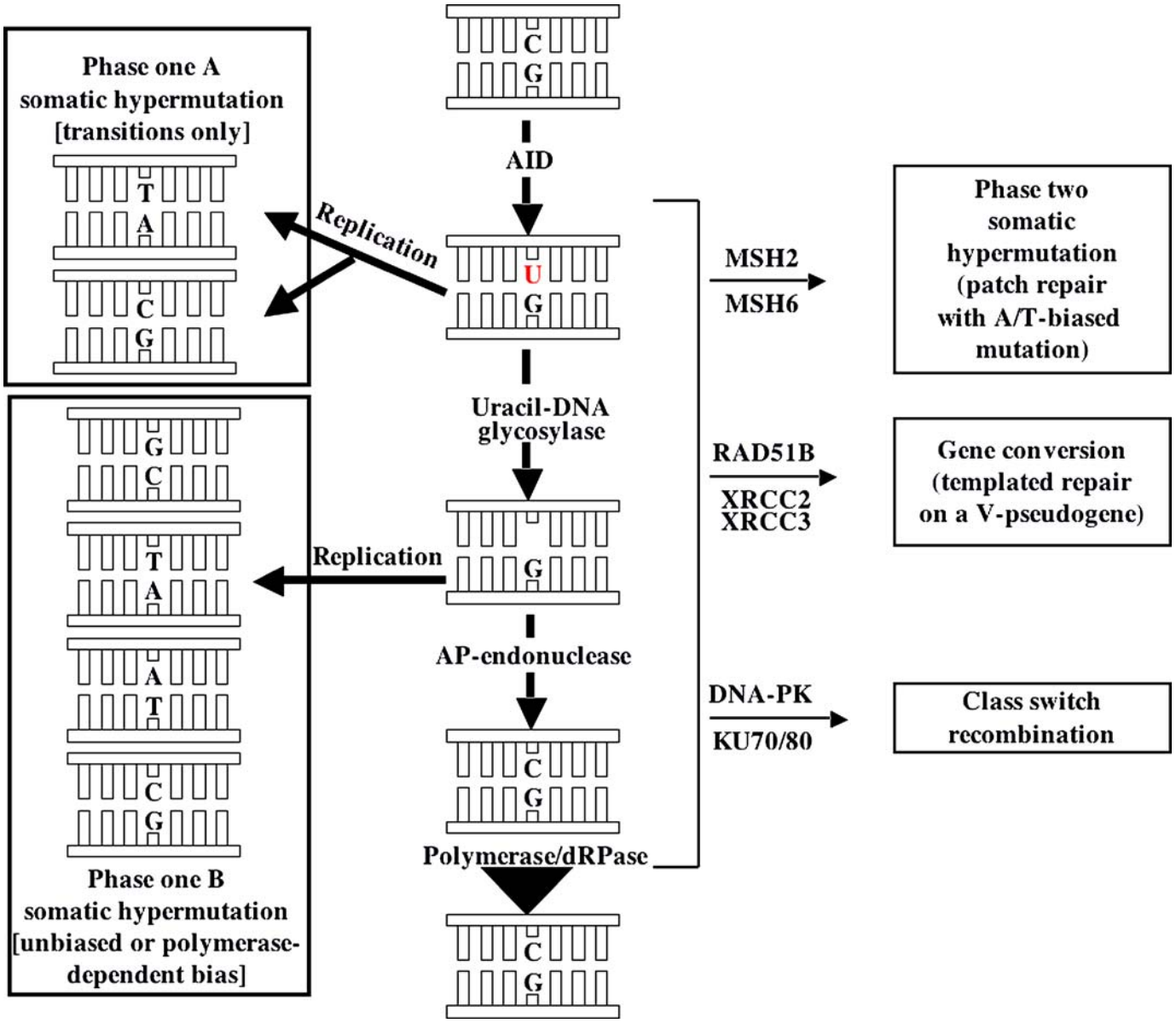

FIGURE 13 DNA deamination model of immunoglobulin diversification. For details see text and Petersen-Mahrt *et al.*, 2002. KU70/80 are non-homologous end-joining proteins. AP endonuclease, apyrimidic endonuclease; DNA-PK, DNA-dependent protein kinase; dRPase, deoxyribophosphodiesterase; V, variable. From Petersen-Mahrt *et al.*, 2002.

hypermutation, which is now attributed to activation-induced cytosine deaminase operating on G:C base pairs (Petersen-Mahrt *et al.*, 2002). In fact, the deamination of cytosine to uracil is apparently the trigger for other forms of post-V-J joining diversification, including gene conversion (Harris *et al.*, 2002b) and class switch recombination (Muramatsu *et al.*, 2000), although this latter pathway is still incompletely understood (see Begum *et al.*, 2004). Figure 13 details the model (Petersen-Mahrt *et al.*, 2002). Replication across the U would result in a G:C → A:T transition, whereas replication across from the AP site generated by excision would result in all base substitutions. Mutations can occur at 5 to 6 orders of magnitude higher levels at the points targeted by somatic hypermutation than for the rest of the genome. AID is a B cell-specific enzyme that is part of a family of enzymes that includes the RNA editing enzyme APOBEC1 (Harris *et al.*, 2002a). Expression of both AID and APOBEC1 in *E. coli* results in a mutator phenotype (Peterson-Mahrt *et al.*, 2002; Harris *et al.*, 2002a). Although this directed mechanism is so far specific to B cell activation (Wang *et al.*, 2004), the concept of inducing an enzyme that generates mutations in a predefined region of the genome is clearly outside the

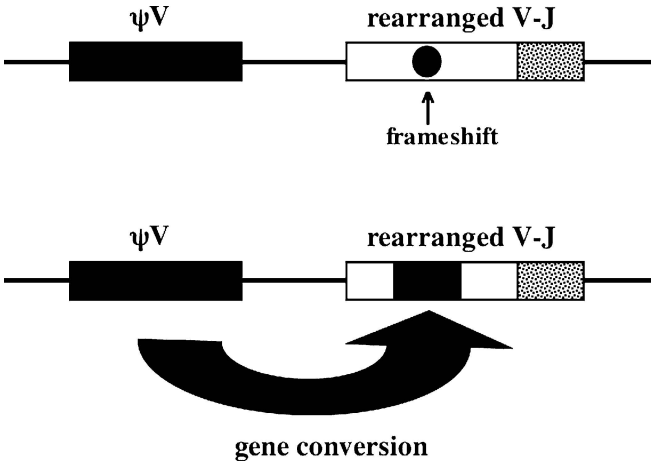

FIGURE 14 **Conceptual diagram of gene conversion in immunoglobulin genes. A psedugene, ψV can contribute sequences to the rearranged V-J gene, in this case reverting a frameshift mutation.**

scope of the Standard Model. Moreover, APOBEC cytosine deaminases are key elements of cellular defenses against infection/invasion by retroelements (reviewed in Turelli & Trono, 2005). For instance, APOBEC3G deaminates dC → dU in minus-strand DNA that is reverse transcribed from HIV RNA, leading to inactivation of functional proviruses (Turelli & Trono, 2005).

Recombination/Gene Conversion From Divergent Sequences

Pseudogenes can serve as templates for gene conversion by allowing recombination between divergent sequences. This mechanism plays a key role in generating antibody diversity in some systems, such as those of chickens and rabbits (Weil & Reynaud, 1992; Harris *et al.*, 2002b). Figure 14 diagrams the concept, and Figure 15 shows an example of sequence changes resulting from gene conversion from pseudogenes. It is not clear how prevalent this mechanism is for spontaneous mutations occurring throughout the genome. In the case of antibody diversity, the process is again triggered by the AID enzyme in B cells (Harris *et al.*, 2002b). Perhaps the recombination events occurring at other pseudogenes are normally kept under check. Any divergent sequence that retains enough homology to be recognized by recombination systems (homologous recombination) is a potential template for mutations. An example is homeologous recombination between *Salmonella* and *E. coli*. In conjugation or transductional crosses, recombination occurs between these two species that are 18% divergent. This recombination is significantly suppressed by a functioning mismatch repair system (Rayssiguier *et al.*, 1989). Although often involving the exchange of large segments, recombination between shorter segments can result in genes that carry sequences from each organism. The *mutS* gene of *E. coli* appears to be a mosaic derived by multiple rounds of recombination with genes from divergent species (Denamur *et al.*, 2000).

Indirect Mutagenesis by Saturation of Mismatch Repair

Like any repair system, the mismatch repair system can be saturated to the point where it can no longer repair additional mismatches. For example, under certain growth conditions, mutants of *E. coli* lacking the epsilon subunit editing function have such high rates of uncorrected mispairs that extracts of cells can no longer repair heteroduplex mismatches (Schaaper, 1988; Damagnez *et al.*, 1989). Mutagens that create lesions or mispairs that are recognized by the mismatch repair system can have the same effect. Thus, a set of base analogs and alkylating agents generate frequent frameshift mutations at repeat tract sequences (often called microsatellites in yeast and higher cells), not by directly targeting

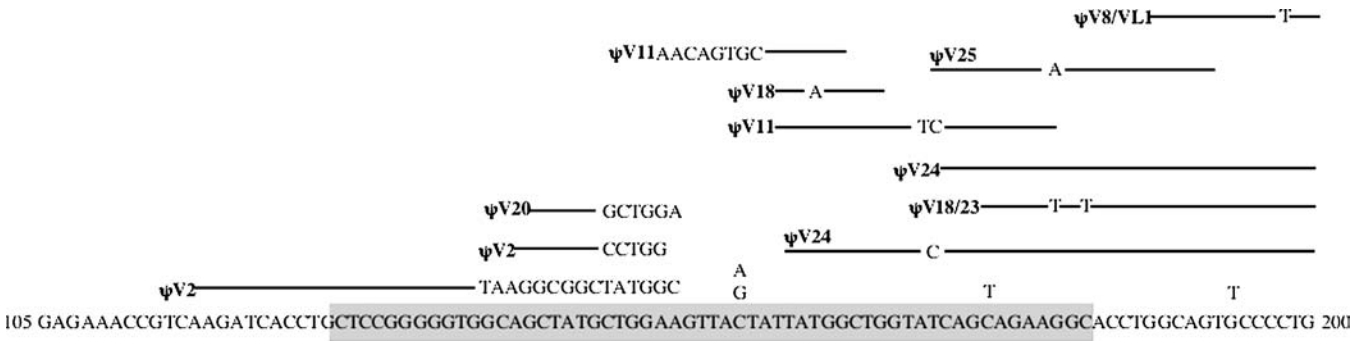

FIGURE 15 **Gene conversion in IgV. Gene conversion and point mutation events are shown in the sequence alignments with the IgV pseudo donors (indicated above the line). From Harris *et al.*, 2002.**

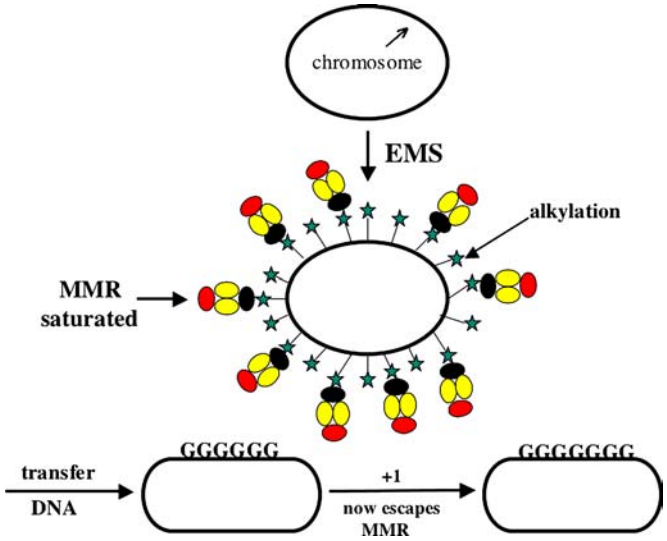

FIGURE 16 Transient saturation of the MMR system. Treatment with EMS causes DNA damage (*stars*) that is recognized by the MutS protein (*closed circles*). Eventual saturation of the MutS proteins allows transferred undamaged DNA to be replicated in the absence of mismatch repair, resulting in frameshifts at repeat-tract sequences. From Miller *et al.*, 2000.

these mutations, but by saturating the mismatch repair system that now can no longer repair the replication errors that occur at these sequences (Cupples *et al.*, 1990; Miller *et al.*, 2000). Figure 16 depicts this mechanism. The fact that these mutagens cause frameshifts indirectly can be shown by examining an unmutagenized target after introduction into mutagen treated cells (Miller *et al.*, 2000; Figure 16). Completely saturating the mismatch repair system will also result in an increase in base substitutions, since replication errors are no longer repaired. However, these mutations are often masked by the mutations directly targeted by the mutagen in question.

Direct Inactivatin of Repair—Cadmium

Cadmium (Cd^{++}) is both toxic and carcinogenic (IARC, 1993; Hengstler *et al.*, 2003), and causes mutations in both yeast and humans by a mechanism that involves direct interaction with a repair systen, rather than with DNA (Jin *et al.*, 2003; reviewed in by McMurray & Tainer, 2003). Cadmium binds to and inactivates the mismatch repair system, thus leading to a large increase in spontaneous mutations, precisely as happens in mismatch repair deficient strains (Jin *et al.*, 2003). Cadmium is thus an example of an indirect-acting mutagen, as depicted in Figure 17. Although certain

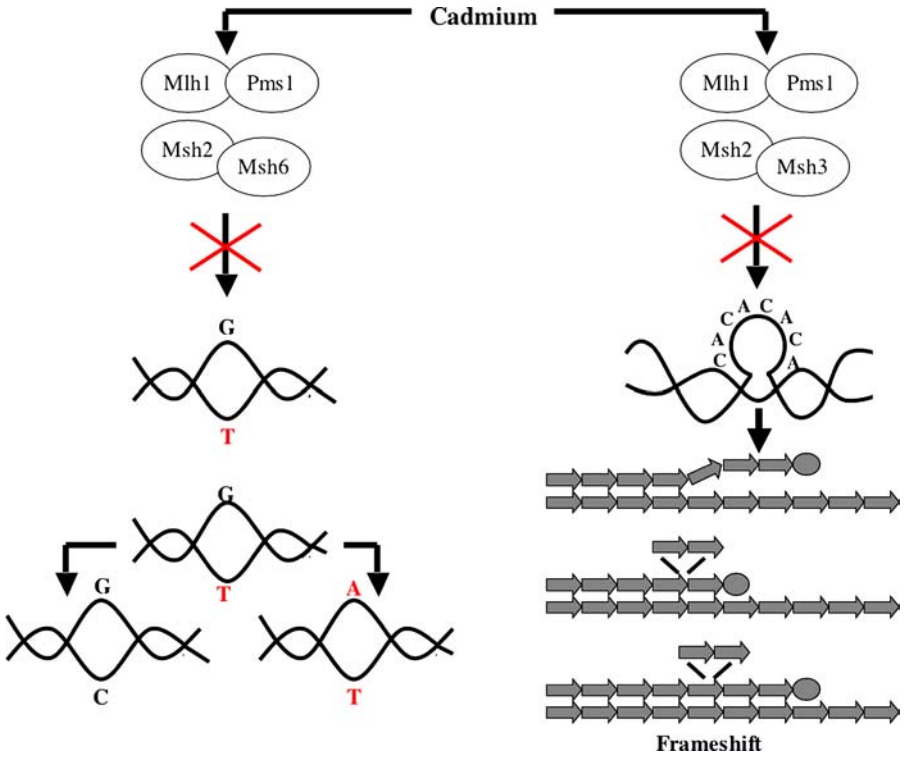

FIGURE 17 Cadmium poisoning of MMR enzymes and resulting mutations. Different heterodimers allow mismatch correction of different types of lesions, as pictured here. Cadmium interferes with the mismatch repair complexes, allowing more mutations of these types to occur, with some of the biological consequences indicated below. From McMurray and Tainer, 2003.

mutagens can induce some mutations indirectly, for instance by making so many lesions in DNA that they saturate the mismatch repair system (see the preceding section), what is distinct about cadmium is that it does not interact with DNA to create lesions, but rather interacts directly with the mismatch repair system itself. It will be interesting to see if other mutagens will be found that inactivate specific repair systems directly.

Repair System Facilitated Mutagenesis

We can now document some cases of repair systems that actually accelerate the rates of certain mutations as a consequence of their activities. For example, normally, oxidized GTP is hydrolyzed to GMP by the MutT repair protein. In the absence of MutT, 8-OxodG gets incorporated across from A, and the action of the MutY glcoslase is primarily responsible for generating mutations by removing the A, allowing repair synthesis to restore a C across from the 8-oxodG, and permitting the MutM glycosylase to remove the 8-oxodG. This results in the original A:T base pair being converted through these steps to a C:G base pair. Strains with increased MutY activity have higher A:T → C:G rates in MutT-deficient backgrounds (Vidmar & Cupples, 1993), and those with inactive MutY have sharply reduced A:T → C:G mutations in MutT-deficient backgrounds (Vidmar & Cupples, 1993; Fowler *et al.*, 2003). Another example of the MutY repair protein actually facilitating mutations is depicted in Figures 18 and 19 (Kim *et al.*, 2003). Because a minor activity of MutY is to remove A from A:C mispairs (Radicella *et al.*, 1988; Michaels *et al.*,1992), MutY competes with the mismatch repair system to resolve A:C mispairs. As Figure 18 shows, when the A:C mispair is derived from an A:T base pair, MutY will create a mutation if it removes the A, since repair synthesis will restore a G across from the C, thus converting an A:T base pair to a G:C base pair. Figure 19 shows how pronounced this effect can be. Here, A:T → G:C transitions at a hotspot site is measured in an NDK (nucleotide diphosphate kinase) deficient strain (black peaks), that has increased A:C mispairing. In a MutY-deficient derivative of this strain, the mutations at this site are virtually eliminated (Kim *et al.*, 2004), showing that MutY is creating these mutations by resolving the A:C mispairs (Figure 18).

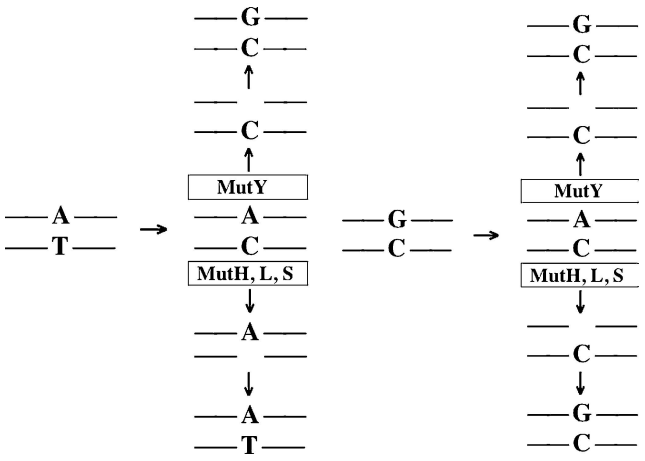

FIGURE 18 **Competition between the MutY protein and the MutHLS MMR system. Left side: Misreplication at an A:T base pair leads to an A:C mispair that is corrected by the MutHLS MMR system but converted to a G:C pair by MutY. Right side: Misreplication at a G:C base pair leads to an A:C mispair that is converted back to a G:C pair by both MutY and the MMR system. (From Kim *et al.*, 2003.)**

Reverse Transcriptase–Tropism Switching

Retroelements, which include retroviruses, are genetic elements that utilize reverse transcriptase (RT) to act on an RNA intermediate during their replication cycle (Doolittle *et al.*, 1989; Xiong & Eickbush, 1990). However, reverse transcription is a highly error-prone process (see Gabriel *et al.*, 1996, and references therein). As a result, retroviruses generate variants at a relatively high rate. This leads, on the one hand, to more frequent resistance to host immune defenses and antiviral drugs, such as seen in HIV *e.g.*, Ritola *et al.*, 2004), but on the other hand, to more rapid mutational decay (Villesen *et al.*, 2004). Thus, human endogenous retroviruses (HERVs), which comprise 1.1% of the human genome, contain mostly degenerate coding sequences (Villesen *et al.*, 2004). Because deleterious mutations are much more frequent than beneficial mutations, strategies involving reverse transcriptase need to be directed to those segments of genes in which hypermutability is beneficial, in analogy to the action of AID on immunoglobulin genes. *Bordetella* bacteriophages use just such a directed mechanism for increasing mutagenesis in a specific region of the gene encoding part of the phage tail fiber (Liu *et al.*, 2002; Doulatou *et al.*, 2004). This strategy involves having a copy of the gene segment replicated by reverse transcriptase in an error-prone manner, before the assembly of the tail fiber gene. For phage Bpp-1, this ensures the generation of diversity

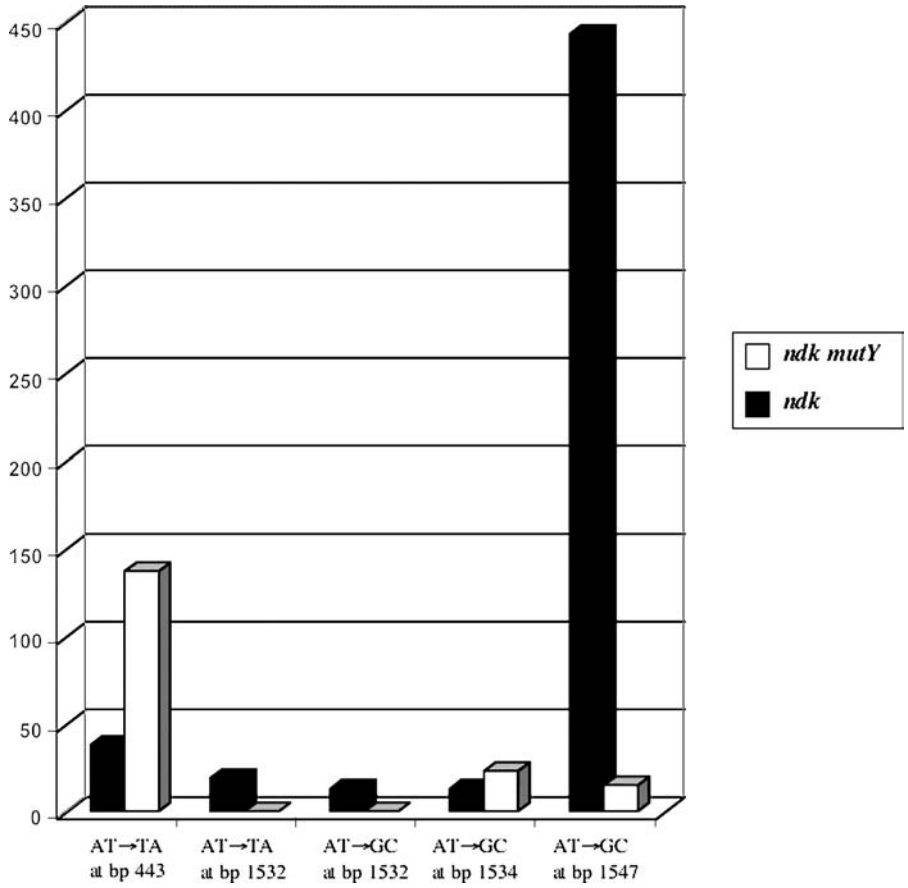

FIGURE 19 **Comparative mutation frequencies in ndk and ndk mutY strains. The frequencies of mutations in the *rpoB* gene at five different sites are shown for both ndk (*white bars*) and ndk mutY (*black bars*) backgrounds. (From Kim *et al.*, 2003.)**

in the gene specifying host tropism, without extensive mutagnesis in the rest of the phage genome. Figure 20 diagrams the sequence of events that allows the definition of a type of diversity generating retroelement (DGR) consisting of a donor template (TR), a recipient of variable seuqneces (VR), and a reverse transcriptase encoding gene (RT) that combine to effect "mutagenic homing" (Doulatov *et al.*, 2004). This type of mechanism may be widespread, since there appear to be a large number of related elements in many different bacteria, including *Nostoc* and *Trichodesmium* species and cyanobacterial species, as well as prophage in *Bordetella* and *Vibrio harveyi* (Doulatov *et al.*, 2004). Interestingly, only A residues are mutated. However, there are 23 A residues in the TR sequence, giving an enormous number of possible mutated VR sequences.

Alternating Repair States of Selected Populations

Selection for fitter cells happens throughout growth and can sometimes lead to mutators overtaking a population, since mutators have higher rates of mutation and subpopulations of mutators have higher frequencies of fitter cells. The stronger the selection, the more rapid is the conversion of a population to all mutators. Figure 21 illustrates an experiment showing how the successive selection for antibiotic resistance to two different antibiotics and a nutritional marker can convert a population of bacteria to all mutators, in this case *mutS*, *mutH*, or *mutL* (Mao *et al.*, 1997), extending earlier work by several groups (Nestman & Hill, 1973; Cox & Gibson, 1974; Chao & Cox, 1983; see also Taddei *et al.*, 1997a, b, and Giraud *et al.*, 2001). When cultures are grown for a very large number of generations in a single media, then a proportion of them (3 of 12) become mutator after several thousand generations (Sniegowski *et al.*, 1997), in response to the weaker but still existing selection for fitter mutants. Yet, continuous growth as a mutator can be counterproductive for a population, since deleterious mutations accumulate (see Funchain *et al.*, 2000; Giraud *et al.*, 2001). Thus, in the absence of selection, non-mutator subpopulations tend to be favored. In fact, the *mutS* gene in *E. coli* can be seen as a mosaic

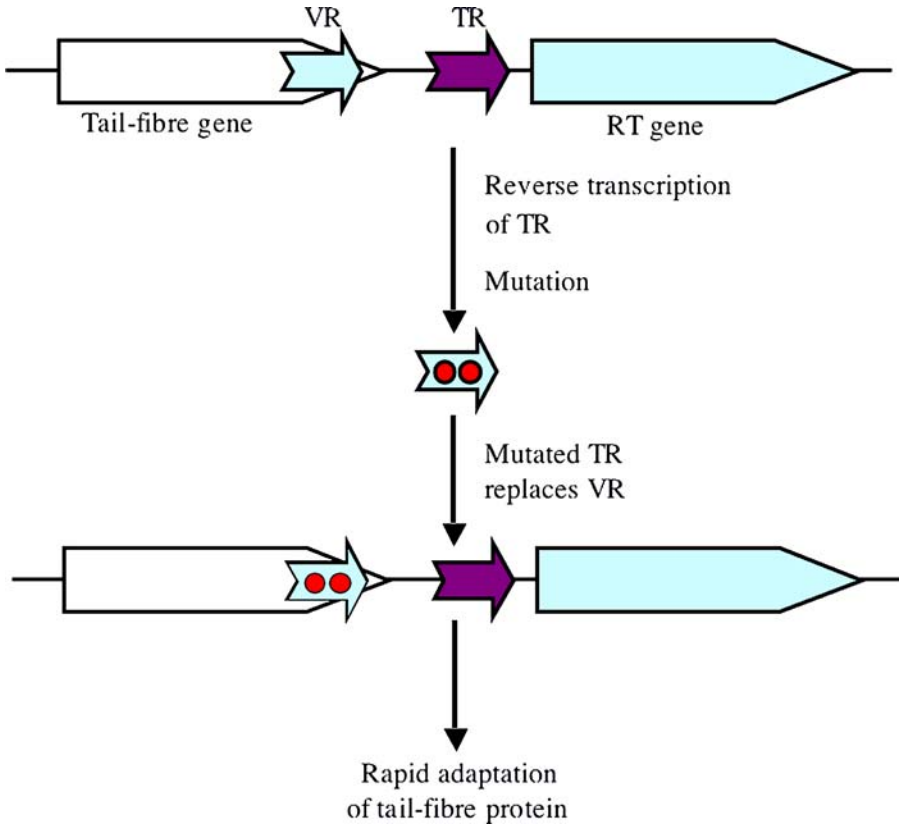

FIGURE 20 **Generating diversity with reverse transcriptase. Top: Bacteriophage BPP-1, a virus that attacks bacteria of the *Bordetella* genus, uses a reverse transcriptase (RT) that copies part of the tail-fibre gene, encoded by the VR and the nearly identical TR segments.. The RT copies the TR segment, itself an invariant source of sequence information, and the frequently mutated copies are then inserted in place of the VR segment. Figure adapted from Boeke, 2004, based on the work of Doulatov *et al.*, 2004.**

of segments derived from different *mutS* genes acquired through rounds of horizontal transfer and recombination (Denamur *et al.*, 2000). Here, horizontal transfer is increased in *mutS* or *mutL* cells (Rayssiguier *et al.*, 1989; Worth *et al.*, 1994), thus selecting for mismatch repair-deficient cells (see Funchain *et al.*, 2001), yet ultimately growth of the population benefits from mismatch repair proficient cells. The alternating requirements over time produce different states of *mutS* that leave footprints (Denamur *et al.*, 2000).

Potential New Additions

Multi-Drug Resistance Pumps

Interestingly, overexpression of the *E. coli emrR* gene, which encodes a regulatory protein for a multi-drug resistance pump, leads to a mutagenic response (Yang *et al.*, 2004), as does overexpression in *E. coli* of the *Pseudomonas aeruginosa nfxB* gene, encoding a *P. aeruginosa* multidrug resistance pump (Grabovsky *et al.*, 2005). In both cases, the mutagenic spectrum suggests replication errors that partially saturate the mismatch repair system. One intriguing possibility is that the failure to synthesize several multi-drug resistance pumps, a consequence of overproduction of a common repressor, prevents the elimination of mutagenic intermediates generated in the normal biosynthesis and metabolism of purines and pyrimidines. At some critical concentration, these compounds might act as base analog mutagens. There are indications that some of the efflux protein pumps may have evolved to reduce toxic levels of metabolic intermediates (Burkovski & Kramer, 2002; Helling *et al.*, 2002; Van Dyk *et al.*, 2004).

Translesion Synthesis by RNA Polymerases: Retromutagenesis

When RNA polymerase transcribes across lesions that have stalled replication, altered mRNAs can be generated, a situation termed "transcriptional mutagenesis" (Doetsch, 1999; Brégeon *et al.*, 2003; reviewed in Doetsch, 2002). These mRNAs can continue to be produced in non-dividing cells. If the resulting altered mRNA or protein confers a growth advantage that

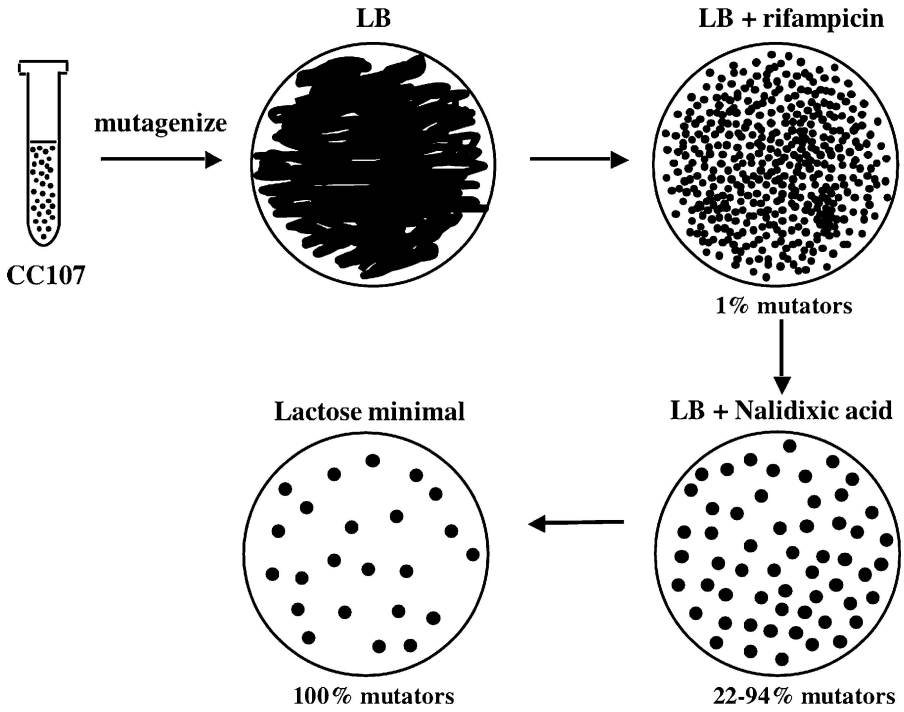

FIGURE 21 The increase in the percentage of mutators in a population of cells undergoing selection. Lac$^-$ cells of strain CC107, containing a frameshift mutation in *lacZ*, are mutagenized with 2AP and are spread onto broth (LB) plates. After overnight growth, the confluent lawn is replicated onto medium with rifampicin, and the grown up resistant mutants are then replicated onto medium with nalidixic acid. The mutants resistant to this antibiotic are then replicated onto lactose minimal medium on which only the Lac$^+$ revertants can grow. Mutators are monitored at each stage. From Mao *et al.*, 1997.

stimulates those cells to start growing, the mutation could be fixed during the first round of replication, provided the DNA lesion also provoked DNA polymerase to make the same base substitution during DNA replication that RNA polymerase made during transcriptional bypass. This would generate a clone of mutants that proliferated as an ultimate result of transcriptional mutagenesis, thus the name retromutagenesis (see Doetsch, 2002, for further details). This type of mechanism has been invoked for starvation-induced mutagenesis (see Bridges, 1994, 1995, 1996).

MUTATION AND CANCER

Cancer involves perturbation of the control of cellular division and the cell cycle (see reviews by Massagué, 2004; Kastan & Bartek, 2004), often by activating oncogenes and by disabling tumor suppressor genes (reviewed in Lowe *et al.*, 2004), and allowing the generation of cancer stem cells (reviewed in Beachy *et al.*, 2004). Is there a single cause of all cancers? Several recent articles consider this question (Marx, 2002; Gibbs, 2003). Much research has focused on the role of mutation and repair in carcinogenesis. The finding that hereditary nonpolyposis colorectal cancer (HNPCC) results from inheriting one defective copy of one of several genes involved in mismatch repair stimulated a lot of work in this area (Fishel *et al.*, 1993; Bhattacharyna *et al.*, 1994; Papadopoulos *et al.*, 1994; Fishel & Kolodner, 1995). It also revived interest in the "mutator" hypothesis (Loeb, 1991, 2001, see commentary by Fishel, 2001, and review by Loeb *et al.*, 2003), that predicts that in order to alter the necessary genes involved in each step toward cancer (now estimated at 5 to 6; see Hahn & Weinberg, 2002), a higher mutation rate is needed. Together with clonal selection for cells with growth advantages (Nowel, 1976; Fishel, 2001), this idea seemed attractive as an explanation for many cancers. However, challenges to this notion come from several directions. On the one hand, as Cairns and others have pointed out, simple mutation cannot explain the origin of the vast majority of cancers (Cairns, 1998, 2002; Thilly, 2003). Rather, it is argued that most cancers result from a stimulation of cell proliferation, allowing the expression of mutations that have accumulated. On the other hand, many cancers have chromosomal abnormalities and changes in number, or aneuploidy. This has led to theories that either the key early events in cancer are mutations (or epigenetic gene silencing; see Klein, 2005) that affect "master genes" controlling cell division and subsequently result

in chromosmomal abnormalities (Lengauer *et al.*, 1998; reviewed in Maser *et al.*, 2002), or that aneuploidy itself is the key early event (Li *et al.*, 1998; Duesberg *et al.*, 1999; reviewed in Rajagopolan & Lengauer, 2004).

Clearly, some cancers do arise from direct mutagenesis, such as UV induced skin cancers (Brash *et al.*, 1991), aflatoxin B1-induced liver cancers (Bressac *et al.*, 1991), or from unrepaired endogenous DNA damage, such as those cancers arising from deficiencies in MutY(MYH)-mediated repair of oxidative damage (Al-Tassan *et al.*, 2002; Jones *et al.*, 2002), or the example of HNPCC cited above. But these may be the exception rather than the rule (Cairns, 1998, 2002). Still, uncovering mutagenic pathways is crucial to understanding routes to carcinogenesis, whether these mechanisms lead to a slow accumulation of mutations that are manifested only during cell proliferation, or whether they result in more direct mutagenesis that affects tumor suppressor genes, oncogenes, or triggers chromosomal abnormalities.

OVERALL PERSPECTIVE AND CONCLUSIONS

As investigators, we look at mutagenesis and repair through a type of experimental prism, separating out each component of the process into defined, simple pathways. However, we find that reconstructing the total blueprint for mutagenesis and repair from our simple analyses remains elusive. We seek different perspectives to give us more viewpoints. Here, we have considered a standard or conventional model with deviations from the model seen as alternative pathways. Figure 1 to 4 and Table 1 summarize the standard model of mutagenesis and repair, and Figures 5 to 21 represent in schematic form the alternative modes of mutagenesis described above. Considering all of these modes, we can see how dynamic the equilibrium is between mutagenesis and avoidance of mutagenesis. Also, this examination helps to define an emerging paradigm, originally suggested by some of the earlier work reviewed here. There are times and places (specific loci) when specific mutagenesis is desired. In some cases, enzyme systems create mutations in defined genes or portions of genes, such as the segments encoding the hypervariable regions of antibodies, and those encoding certain phage tail fibers or bacterial surface antigens (tropism switching). Sometimes gene conversion is used to create variation in a portion of a gene. Some genes have mutation prone sequences built in to increase variability, as is found for segments of certain genes encoding parts of surface antigens, so-called contingency loci. Under stress, cells or subpopulations of cells. may experience increased mutagenesis to allow either the bypass of replication blocks or the selection of new variants. On the other hand, mutation avoidance and repair systems are essential for preventing unwanted mutagenesis, providing just the right balance of forces.

Mutation and recombination are often seen as part of the basic set of tools that drive evolution (e.g. see reviewed in Radman *et al.*, 2000), along with mobile elements that in some cases constitute a significant fraction of genomes (reviewed in Kazazian, 2004) and contribute to the evolution of antibiotic resistance cassettes (reviewed in Maizel, 2004). Supplementing these mechanisms is horizontal transfer (see volume by Bushman, 2002), which some argue is a major contributor to genome evolution (Brown, 2003; Doolittle, 1999; Ochman *et al.*, 2000; Koonin, 2003; Lawrence & Hendrickson, 2003), although others caution about the pitfalls in attributing the origin of portions of genomes to horizontal gene transfer (Eisen & Fraser, 2003). Alternatively, the combination of symbiosis, cooperation, and the acquisition of genomes has been suggested as the major force driving evolution (Margulis & Sagan, 2002).

What role does the set of alternate mechanisms described in this review play in evolution? Clearly, the enzymatic stimulation of mutations affecting antibody hypervariable regions and tropism switching are programmed mechanisms for increasing diversity, as are the DNA sequenced-programmed contingency loci. Also, subpopulations of mutators can be dramatically increased in response to selection for specific mutants, because of their greater propensity to create favorable variants. But what about more general increases in mutation level such as seen in the induction of SOS or of other stress responses such as adaptive mutagenesis? It has been argued that the extra mutations, seen for example after induction of the SOS system, are involved in driving evolution (*e.g.*, Radman, 1999; Radman *et al.*, 2000), although it is not certain that these mutations are not simply the unwanted consequences of replication bypass of a blocking lesion, namely, the price to pay for survival in dire circumstances.

Remarkably, most of what we know about mutagenesis and repair comes from the study of only a few well-characterized organisms. As genomics and related fields

allow us to examine mutational pathways and avoidance mechanisms in microorganisms living in different and sometimes extreme environments, we will no doubt encounter many new phenomena. Perhaps, we shall describe these findings as Howard Carter did when he discovered Tutankhamun's tomb (Carter, 1922). When asked by his co-excavators, "Can you see anything?" He replied, "Yes, wonderful things."

ACKNOWLEDGEMENT

The Author was supported by a grant from the National Institutes of Health (grant no. ES0110875).

REFERENCES

Anderson, R.P. and Roth, J.R. 1978. Tandem genetic duplications in *Salmonella typhimurium*: amplification of the histidine operon. *J Mol Biol* 126:53–71.

Andersson, D.I., Slechta, E.S., and Roth, J.R. 1998. Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon. *Science* 282:1133–1135.

Al-Tassan, N., Chmiel, N.K., Maynard, J., Fleming, N., Livingston, A.L., Williams, G.T., Hodges, A.K., Davies, D.R., David, S.S., Sampson, J.R., and Cheadle, J.P. 2002. Inherited variants of *MYH* associated with somatic G:C→T:A mutations in colorectal tumors. *Nature Genet* 30: 227–232.

Bhattacharyya, N.P., Skandalis, A., Ganesh, A., Groden, J., and Meuth, M. Mutator phenotypes in human colorectal carcinoma cell lines. Proc. *Natl Acad Sci USA* 91:6319–6323.

Balashov, S. and Humayun, M.Z. 2004. Specificity of spontaneous mutations induced in *mutA* mutator cells. 2004. *Mutation Res* 54:9–18.

Barnes, D.E. and Lindahl, T. 2004. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* 38:445–476.

Bayliss, C.D., Dixon, K.M., and Moxon, E.R. 2004a. Simple sequence repeats (microsatellites): mutational mechanisms and contributions to bacterial pathogenesis. A meeting review. *FEMS Immun Med Microbiol* 40:11–19.

Bayliss, C.D., Sweetman, W.A., and Moxon, R.E. 2004b. Mutations in *Haemophilus influenzae* mismatch repair genes increase mutation rates of dinucelotide repeat tracts but not dinucleotide repeat-driven pilin phase variation rates. *J Bacteriol* 186:2928–2935.

Bayliss, C.D., Field, D., and Moxon, E.R. 2001. The simple sequence contingency loci of *Haemophilus influenzae* and *Neisseria meningitides J Clin. Invest* 107:657–662.

Beachy, P.A., Karhadkar, S.S., and Berman, D.M. 2004. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 432:324–331.

Bellacosa, A. 2001. Functional interactions and signaling properties of mammalian DNA mismatch repair protein. *Cell Death and Differentiation* 8:1076–1092.

Begum, A.A., Kinoshita, K., Kakazu, N., Muramatsu, M., Nagoaka, H., Shinkura, R., Biniszkiewicz, D., Boyer, L.A., Jaenisch, R., and Honjo, T. 2004. Uracil DNA Glycosylase Activity is dispensable for immunoglobulin class switch. *Science* 305:1160–1163.

Boeke, J.D. 2004. A is for adaptation. *Nature* 431:408–409.

Brash, D.E., Rudolph, J.A., Simon, J.A., Lin, A., McKenna, G.J., Baden, H.P., Halperin, A.J., and Ponten, J. 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 88:10124–10128.

Brégeon, D., Doddridge, Z.A., You, H.J., Weiss, B., and Doetsch, P.W. 2003. Transcriptional mutagenesis induced by uracil and 8-oxoguanine in *Escherichia coli Molec Cell* 12:959–970.

Bressac, B., Kew, M., Wands, J., and Ozturk, M. 1991. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern frica. *Nature* 350:429–231.

Bridges, B.A. 1994. Starvation-associated mutation in *Escherichia coli*: a spontaneous lesion hypothesis for "directed" mutation. *Mutat Res* 307:149–156.

Bridges, B.A. 1995. MutY "directs" mutation? *Nature* 375:741.

Bridges, B.A. 1996. Mutation in resting cells: the role of endogenous DNA damage. *Cancer Surv* 28:155–167.

Brown, J.R. 2003. Ancient horizontal gene transfer. *Nat Rev Genet* 4:121–132.

Burkovski, A. and Krämer, R. 2002. Bacterial amino transport proteins: occurrence, functions, and significance for biotechnological applications. *Appl Microbiol Biotechnol* 58:265–274.

Bushman, F. 2002. Lateral DNA transfer: Mechanisms and consequences. Cold Spring Harbor Laboratory Press. pp. 1–448.

Cadmium IARC. 1993. Monographs on the Evaluation of Carcinogenic Risks to Humans119–237 (IARC, Lyon, France).

Cairns, J. 1998. Mutation and cancer: The antecedents to our studies of adaptive mutation. *Genetics* 128:1433–1440.

Cairns, J. 2002. Somatic stem cells and the kinetics of mutagenesis and carcinogenesis. *Proc Natl Acad Sci USA* 99:10567–10570.

Cairns, J., Overbaugh, J., and Miller, S. 1988. The origin of mutants. *Nature* 335:142–145.

Caillet-Fauquet, P. and Maenhaut-Michel, G. 1988. *Mol Gen Genet* 213:491– 498.

Carter, H. 1922. Notes, Diary, and Articles, referring to the Thebian royal Necropolis and the Tomb of Tutankhamen. *Griffith Institute Archive* (TAA Archive i.2.1)., Griffith Institute, Oxford, England.

Chao, L. and Cox, E.C. 1983. Competition between high and low mutating strains *of Escherichia coli Evolution* 37:125–134.

Courcelle, J., Donaldson, J.R., Chow, K.-H., and Courcelle, C.T. 2004. DNA damage-induced replication fork regression and processing in *Escheirichia coli Science* 299:1064–1067.

Cox, E.C. and Gibson, T.C. 1974. Selection for high mutation rates in chemostats. *Genetics* 77:169–184.

Cox, E.C. and Horner, D.L. 1982. Dominant mutators in *Escherichia coli Genetics* 100:7–18.

Cupples, C.G., Cabrera, M., Cruz, C., and Miller, J.H. 1990. A set of *lacZ* mutations in *Escherichia coli* that allows rapid detection of specific frameshift mutations. *Genetics* 125:275–280.

Damagnez, V., Doutriaux, M.-P., and radman, M. 1989. Saturation of mismatch repair in the *mutD5* mutator strain of *Escherichia coli*. *J Bacteriol* 171:4494–4497.

De Boer, J.G. and Ripley, L.S. 1984. Demonstration of the production of frameshift and base-substitution mutations by quasipalindromic DNA sequences. *Proc Natl Acad Sci USA* 81:5528–5531.

Degnen, G.E. and Cox, E.C. 1974. Conditional mutator gene in *Escherichia coli*: isolation, mapping, and effector studies. *J Bacteriol* 117:477–487.

Denamur, E., Lecointre, G., Dariu, P., Tenaillon, O., Acquaviva, C., Sayada, C., Sunjevaric, I., Rothstein, R., Elion, J., Taddei, F., Radman, M., and Matic, I. 2000. Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. *Cell* 103:711–721.

Doetsch, P.W. 2002. Translesion synthesis by RNA polymerases: occurrence and biological implications for transcriptional mutagenesis. *Mutat Res* 510:131–140.

Doetsch, P.W., Viswanathan, A., Zhou, W., and Liu, J. 1999 . Bypass of DNA damage by RNA polymerases: implication for DNA repair and transcriptional mutatgenesis in advances in DNA damage and repair. Dizdaraglu, M. and Karakaya, A. eds. New York, Plenum Publishers. pp. 97–109.

Doolittle, R.F., Feng, D.F., Johnson, M.S., McClure, M.A. 1989. Origins and evolutionary relationships of retroviruses. *Q Rev Biol* 64:1–30.

Doolittle, W.F. 1999. Phylogenetic classification and the universal tree. *Science* 284:2124–2129.

Doulatov, S., Hodes, A., Dai, L., Mandhana, N., Liu, M., Deora, R., Simons, R.W., Zimmerly, S., and Miller, J.F. 2004. Tropism switching

in *Bordetella* bacteriophage defines a family of diversity-generating retroelements. *Nature* 431:476–481.
Drake, J.W. 1991. A constant rate of spontaneous mutation in DNA-based microbes. *Proc Natl Acad Sci USA* 88:7160–7164.
Drake, J.W., Charlesworth, B., Charlesworth, D., and Crow, J.F. 1998. Rates of spontaneous mutation. *Genetics* 148:1667–1686.
Duesberg, P., Rasnick, D., Li, R., Winters, L., Rausch, C., and Hehlmann, R. 1999. How aneuploidy may cause cancer and genetic instability. *Anticancer Res* 19:4887–4906.
Echols, H. and Goodman, M.F. 1991. Fidelity mechanisms in DNA replication. *Annu Rev Biochem* 60:477–511.
Echols, H., Lu, C., and Burgers, P.M.J. 1983. Mutator strains of *Escherichia coli*, *mutD5* and *dnaQ*, with defective exonucleolytic editing by DNA polymerase III holoenzyme. *Proc Natl Acad Sci* USA 80:2189–2192.
Edlund, T., and Normark, S. 1981. Recombination between short DNA homologies causes tandem duplication. *Nature* 292:269–271.
Eisen, J.A. and Fraser, C.M. 2003. Phylogenomics: intersection of evolution and genomics. *Science* 300:1706–1707.
Falnes, P.O., Johansen R.F., and Seeberg E. 2002. AlkB-mediated oxidative demethylation reverses DNA damage in *Escherichia coli*. *Nature* 419:178–181.
Farabaugh, P.J., Schmeissner, U., Hofer, M., and Miller, J.H. 1978. Genetic studies of the *lac* repressor. VII. On the molecular nature f spontaneous hotspots in the *lac*I gene of *Escherichia coli*. *J Mol Biol* 126:847–863.
Fijalkowska, I.J., Dunn, R.L., and Schaaper, R.M. 1997. Genetic requirements and mutational specificity of the *Escherichia coli* SOS mutator activity. *J Bacteriol* 179:7435–7445.
Fishel, R. 2001. The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. *Cancer Res* 61:7369–7374.
Fishel, R. and Kolodner, R.D. 1995. Identification of mismatch repair genes and their role in the development of cancer. *Curr Opin Genet Dev* 5:382–395.
Fishel, R., Lescoe, M.K., Rao, M.R., Copelan, N.G., Jenkins, N.A., Garber, J., Kane, M., and Kolodner, R. 1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell* 75:1027–1038.
Foster, P.L. 2004a. Rebuttal: Adaptive point mutation (Rosenberg and Hastings). *J Bacteriol* 186: 4845.
Foster, P.L. 2004b. Adaptive mutation in *Escherichia coli J Bacteriol* 186:4846–4852.
Foster, P.L. 2004c. Rebuttal: Growth under selection stimulates Lac^+ reversion (Roth and Andersson). *J Bacteriol* 186:4861.
Foster, P.L., and Cairns, J. 1992. Mechanisms of directed mutation. *Genetics* 131:783–789.
Fowler, R.G., White, S.J., Koyama, C., Moore, S.C., Dunn, R.L., and Schaaper, R.M. 2003. Interactions among the *Escherichia coli mutT*, *mutM*, and *mutY* damage prevention pathways. *DNA Repair* 2:159–173.
Friedberg, E.C. 2003. DNA damage and repair. *Nature* 421:436–440.
Friedberg, E.C., McDaniel, L.D., and Schultz, R.A. 2004. The role of endogenous and exogenous DNA damage and mutagenesis. *Curr Opin Genet Dev* 14:5–10.
Friedberg E.C., Wagner, R., Radman, M. 2002. Specialized DNA polymerases, cellular survival, and the genesis of mutations. *Science* 296:1627–1630.
Friedberg, E.C., Walker, G.C., and Seide, W. 1995. *DNA Repair and Mutagenesis.* Washington, D.C., American Society for Microbiology, p. 638.
Funchain, P., Yeung, A., Stewart, J.L., Lin, R., Slupska, M.M., and Miller, J.H. 2000. The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. *Genetics* 154:959–970.
Funchain, P., Yeung, A., Stewart, J., Clendenin, W., and Miller, J.H. 2001. Amplification of mutator cells in a population as a result of horizontal transfer. *J Bacteriol* 183:3737–3741.
Gabriel, A., Willems, M., Mules, E.H., and Boeke, J.D. 1996. Replication infidelity during a single cycle of *Ty1* retrotransposition. *Proc Natl Acad Sci USA* 93:7767–7771.
Garibyan, L., Huang, T., Kim, M., Wolff, E., Nguyen, A., Nguyen, T., Diep, A., Hu, K., Iverson, A., Yang, H., and Miller, J.H. 2003. Use of the rpoB gene to determine the specificity of base substitution mutations on the *Escherichia coli* chromosome. *DNA Repair (Amst)* 2:593–608.
Gibbs, W.W. 2003. Untangling the roots of cancer. *Sci American* 289:56–65.
Giraud, A., Matic, I., Tenaillon, O., Clara, A., Radman, M., Fons, M., and Taddei, F. 2001. Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. *Science* 291:2606–2608.
Gordenin, D.A., Kunkel, T.A., and Resnick, M.A. 1997. Repeat expansion—All in a flap? *Nature Genet* 16:116–118.
Grabovsky, V., Yamamoto, M.L., and Miller, J.H. 2005. Mutator effects in *Escherichia coli* caused by the expression of specific foreign genes. *J. Bacteriol* (In press).
Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R.C., and Gelbart, W.M. 1999. *An Introduction To Genetic Analysis, 7th ed.* New York, W. H. Freeman.
Guarné, A., Ramon-Maiques, S., Wolff, E.M., Ghirlando, R., Hu, X., Miller, J.H., and Yang, W. 2004. *EMBO J* 23:4134–4145.
Hahn, W.C. and Weinberg, R.A. 2002. Rules for making human tumor cells. *N Engl J Med* 347:1593–1603.
Hall, B.G. 1997. On the specificity of adaptive mutations. *Genetics* 145:39–44.
Harris, R.S., Petersen-Mahrt, S.K., and Neuberger, M.S. 2002a. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. *Molec Cell* 10:1247–1253.
Harris, R.S., Sale, J.E., Petersen-Mahrt, S.K., and Neuberger, M.S. 2002b. AID is essential for immunoglobulin V gene conversion in a cultured B cell line. *Curr Biol* 12:435–438.
Hastings, P.J., Bull, H.J., Klump, J.R., and Rosenberg, S.M. 2000. Adaptive amplification: an inducible chromosomal instability mechanism. *Cell* 103:723–731.
Helling, R.B., Janes, B.K., Kimball, H., Tran, T., Bundesmann, M., Check, P., Phelan, D., and Miller, C. 2002. Toxic waste disposal in *Escherichia coli. J Bacteriol* 184:3699–3703.
Hengstler, J.G., Bolm-Audorff, U., Faldum, A., Janssen, K., eifenrath, M., Gotte, W., Jung, D., Mayer-Popken, O., Fuchs, G., Gebhard, S., Bienfait, H. G., Schlink, K., Dietrich, C., Faust, D., Epe, B., and Oesch, F. 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangersous than hitherto expected. *Carcinogenesis* 24:63–73.
Hendrickson, H., Schlechta, E.S., Bergthorsson, U., Andersson, D.I., and Roth, J.R. 2002. Amplification mutagenesis: evidence that 'directed' adapative mutation and general hypermutability result from growth with a selected gene amplification. *Proc Natl Acad Sci USA* 99:2164–2169.
Horiuchi, T., Horiuchi, S., and Novick, A. 1963. The geneic basis of hypersynthesis of beta-galactosidase. *Genetics* 48:157–169.
Humayun, M.Z. 1998. SOS and Mayday: multiple inducible mutagenic pathways *in Escherichia coli. Mol Microbiol* 30:905–910.
Jin, Y.H., Clark, A.B., Slebos, R.J.C., Al-Refai, H., Taylor, J.A., Kunkel, T.A., Resnick, M.A., and Gordenin, D.A. 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nature Genet* 34:326–329.
Jones, S., Emmerson, P., Maynard, J., Best, J.M., Jordan, S., Williams, G.T., Sampson, J.R., and Cheadle, J.P. 2002. Biallelic germline mutations in *MYH* predispose to multiple colorectal adenoma and somatic G:C → T:A mutations. *Hum Molec Genetics* 11:2961–2967.
Judson, H.F. 1979. *The Eighth Day of Creation*, New York, Simon and Schuster, 686.
Kane, G. 2003. The dawn of physics beyond the standard model. *Sci American* 288:68–75.

Kang, S., Jaworski, A., Ohshima, K., and Wells, R.D. 1995. Expansion and deletion of CTG repeats from human disease genes are determined by the direction of replication in *E coli Nat Genet* 10:213–218.
Kastan, M.B. and Bartek, J. 2004 . Cell-cycle checkpoints and cancer. *Nature* 432:316–323.
Kazazian, H.H. Jr. 2004. Mobile elements: drivers of genome evolution. *Science* 303:1626–1632.
Kim, M., Huang, T., and Miller, J.H. 2003. Competition between MutY and Mismatch repair at A:C mispairs in vivo. *J Bacteriol* 185:4626–4629.
Kim, S.R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T., and Ohmori, H. 1997. Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. *Proc Natl Acad Sci USA* 94:13792–13797.
Klein, G. 2005. Surveillance team against cancer. *Nature.* 434: 150.
Kolodner, R. 1996. Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev* 10:1433–1442.
Koonin, E.V. 2003. Horizontal gene transfer: the path to maturity. *Mol Microbiol* 50:725–727.
Kornberg, A. and Baker, T. 1992. *DNA Replication*, 2d ed. New York, W.H. Freeman, p. 931.
Lawrence, J.G. and Hendrickson, H. 2003. Lateral gene transfer: when will adolescence end? *Mol Microbiol* 50:739–749.
Layton, J.C. and Foster, P.L. 2003. Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli*. *Molec Microbiol* 50:549–561.
Leach, F.S., Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L.A., Nystron-Lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P.S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K.M., Fournier, R.E.K., Todd, S., Lewis, T., Leach, R. J., Naylor, S.L., Weissenbach, J., Meckin, J.-P., Jarvinen, H., Petersen, G.M., Hamilton, S.R., Green, J., Jass, J., Watson, P., Lynch, H.T., Trent, J.M., de la Chapelle, A., Kinsler, K.W., and Vogelstein, B. 1993. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75:1215–1225.
Lengauer, C., Kinzler, K.W., and Vogelstein, B.E. 1998. Genetic instabilities in human cancers. *Nature* 396:643–649.
Li, R., Sonik, A., Stindl, R., Rasnik, D., and Duesberg, P. 2000. Aneupoidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. *Proc Natl Acad Sci USA* 97:3236–3241.
Lindahl, T. 2001. Keynote: past, present, and future aspects of base excision repair. *Prog Nucleic Acid Res Mol Biol* 68:xvii–xxx.
Lindahl, T. and Barnes, D.E. 2000. Repair of endogenous DNA damage. *Cold Spring Harb Symp Quant Biol* 65:127–133.
Liu, M., Deora, R., Doulatov, S.R., Gingery, M., Eiserling, F.A., Preston, A., Maskell, D.J., Simons, R.W., cotter, P.A., Parkhill, J., and Miller, J.F. 2002. Reverse transcriptase-mediated tropism switching in *Bordetella* bacteriophage. *Science* 295:2091–2094.
Loeb, L.A. 1991. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res* 51:3075–3079.
Loeb, L.A. 2001. A mutator phenotype in cancer. *Cancer Res.* 61:3230–3251.
Loeb, L.A., Loeb, K., and Anderson, J.P. 2003. Multiple mutations and cancer. *Proc Natl Acad Sci USA* 100:776–781.
Lombardo, M.J., Aponyi, I., and Rosenberg, S.M. 2004. General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli*. *Genetics* 166:669–680.
Lovett, S. 2004. Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences. *Molec Microbiol* 52:1243–1253.
Lowe, S.W., Cepero, W., and Evan G. 2004. Intrinsic tumor suppression. *Nature* 432:307–315.
McMurray, C.T., and Tainer, J.A. 2003. Cancer, cadmium and genome integrity. *Nature Genet* 34:239–241.
Maizel, D. 2004. Integrons and the origin of antibiotic resistance gene cassettes.*ASM News* 70:520–525.
Mao, E.F., Lane, L., Lee, J., and Miller, J.H. 1997. Proliferation of mutators in a cell population. *J Bacteriol* 179:417–422.
Margulis, L. and Sagan, D. 2002. *Acquiring Genomes*: *A Theory of the Origin of Species.* New York, NY: Basic Books, p. 256.
Martin, P., Sun, L., Hood, D.W., and Moxon, E.R. 2004. Involvement of genes of genome maintenance in the regulation of phase variation frequencies in Neisseria meningitides. *Microbiol* 150:3001–3012.
Maruyama, M., Horiuchi, T., Maki, M., and Sekiguchi, M. 1983. A dominant (*mutD5*) and a recessive (*dnaQ49*) mutator of *Escherichia coli*. *J Mol Biol* 167:751–771.
Marx, J. 2002. Debate surges over the origins of genomic defects in cancer. *Science* 297:544–546.
Maser, R.S. and DePinho, R.A. 2002. Connecting chromosomes, crisis and cancer. *Science* 297:565–569.
Massagué, J. 2004. G1 cell-cycle control and cancer. *Nature* 432:298–306.
Michaels, M.L., Cruz, C., and Miller, J.H. 1990. *mutA* and *mutC*: two mutator loci in *Escherichia coli* that stimulate transversions. *Proc Natl Acad Sci USA* 87:9211–9215.
Michaels, M.L., Tchou, J., Grollman, A.P., and Miller, J.H. 1992. A repair system for 8-oxo-7,8-dihydrodeoxyguanine. *Biochemistry* 31:10964–10968.
Miller, J.H. and Low, K.B. 1984. Specificity of mutagenesis resulting from the induction of the SOS system in the absence of mutagenic treatment. *Cell* 37:675–682.
Miller, J.H., Yeung, A., Funchain, P., Mao, E., Stewart, J., Clendenin, W. M., and Slupska, M.M. 2000. Temporary and permanent mutators lacking the mismatch repair system: the enhancement of mutators in cell populations. *Cold Spring Harbor Symp Quant Biol* LXV:241–252.
Mirkin, S. and Smirnova, E. 2002. Positioned to expand. *Nat Genet* 31:5–6.
Modrich, P. 1991. Mechanisms and biological effects of mismatch repair. *Annu Rev Genet* 25:229–253.
Modrich, P. and Lahue, R. 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu Rev Biochem* 65:101–133.
Moxon, E.R., Rainey, P.B., Nowak, M.A., and Lenski, R.E. 1994. Adaptive evolution of highly mutable loci in pathogenic bacteria. *Curr Biol* 4:24–33.
Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Tasuku, H. 2000. Class switch recombination and hypermutation require activation induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102:553–563.
Murphy, H.S. and Humayun, M.Z. 1997. *Escherichia coli* cells expressing a mutant *glyV* (glycine tRNA) gene have a UVM-constitutive phenotype: implications for mechanisms underlying the *mutA* or *mutC* mutator effect. *J Bacteriol* 197:7507–7514.
Nestman, E.R. and Hill, R. F. 1973. Population changes in continuously growing mutator cultures of *Escherichia coli*. *Genetics* 73(Suppl.):41–44.
Ninio, J. 1991. Transient mutators: a semiquantitative analysis of the influence of translation and transcription errors on mutation rates. *Genetics* 129:957–962.
Noia, J.D. and Neuberger, M.S. 2002. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. *Nature* 419:43–48.
Novichkov, P.S., Omelchenko, M.V., Gelfand, M.S., Mironov, A.A., Wolf, Y.I., and Koonin, E.V. 2004. Genome-wide molecular clock and horizontal gene transfer in bacterial evolution. *J Bacteriol* 186:6575–6585.
Nowell, P.C. 1976. The clonal evolution of tumor populations. *Science* 194:23–28.
Ochman, H., Lawrence, J.G., and Groisman, E.A. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405:299–304.

Ohmori, H., Friedberg, E.C., Fuchs, R.P.P., Goodman, M.F., Hanaoka, F., Hinkle, D., Kunkel, T.A., Lawrence, C.W., Livneh, Z., Nohmi, T., Prakash, S., Todo, T., Walker, G.C., Wang, Z., and Woodgate, R. 2001. The Y-family of Dna polymerases. *Mol Cell* 8:7–8.

Okazaki, L, Kinoshita, K., Muramatsu, M, Yoshikawa, K., and Honjo, T. 2002. The AID enzyme induces class switch recombination in fibroblasts. *Nature* 416:340–345.

Papadopoulos, N., Nicolaides, N.C., Wei, Y.F., Ruben, S.M., Carter, K.C., Rosen, C.A., Haseltine, W.A., Fleischman, R.D., Fraser, C.M., Adams, M.D., Venter, J.C., Hamilton, S.R., Petersen, G.M., Watson, P., Lynch, H.T., Peltomaki, P., Mecklin, J.-P., de la Chapelle, A., and Vogelstein, B. 1994. Mutation of a *mutL* homolog in hereditary colon cancer. *Science* 263:1625–1629.

Petersen-Mahrt, S.K., Harris, R.S., and Neuberger, M.S. 2002. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* 418:99–103.

Radicella, J.P., Clark, E.A., and Fox, M.S. 1988. Some mismatch repair activities in *Escherichia coli Proc Natl Acad Sci USA* 85:9674–9678.

Radman, M. 1974. Phenomenology of an induction mutagenic DNA repair pathway in *Escherichia coli*: SOS repair hypothesis. In: *Molecular and environmental Aspects of Mutagenesis*, Prakash, L., Sherman, F., Miller, M., Lawrence, C., and Tabor, H.W. eds. Springfield, IL, Charles C. Thomas, Publisher. 128–142.

Radman, M. 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis. In: *Molecular Mechanisms for Repair of DNA, Part A*. Hanawalt, P., and Setlow, R.B. eds. Plenum Publishing Corp. New York, 355–367.

Radman, M. 1999. Mutation: enzymes of evolutionary change. *Nature* 401:866–869.

Radman, M., Taddei, F., and Matic, I. 2000. Evolution-driving genes. *Res Microbiol* 151:91–95.

Rajagopalan, H. and Lengauer, C. 2004. Aneuploidy and cancer. *Nature* 432:338–341.

Rayssiguier, C., Thaler, D.S., and Radman, M. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch repair mutants. *Nature* 342:396–401.

Reuven, N.B., Arad, G., Maor-Shoshani, A., and Livneh, Z. 1999. *J Biol Chem* 274:31763–31766.

Richard, G., Hennequin, C., Thierry, A., and Dujon, B. 1999. Trinucleotide repeats and other microsatellites in yeasts. *Res Microbiol* 150:589–602.

Ripley, L.S. 1982. Model for the participation of quasi-palindromic DNA sequences in frameshift mutation. *Proc Natl Acad Sci USA* 79:4128–4132.

Ritola, K., Pilcher, C.D., Fiscus, S.A., Hoffman, N.G., Nelson, J.A., Kitrinos, K.M., Hicks, C.B., Eron, J.J. Jr., and Swanstrom, R. 2004. Multiple V1/V2 env variants are frequently present during primary infection with human immunodeficiency virus type 1. *J Virol* 78:11208–11218.

Rosenberg, S.M. and Hastings, P.J. 2004a. Adaptive point mutation and adaptive amplification pathways in the *Escherichia coli* Lac system: Stress responses producing genetic change. *J Bacteriol* 186:4838–4843.

Rosenberg, S.M. and Hastings, P.J. 2004b. Rebuttal: Adaptive mutation in *Escherichia coli* (Foster). *J Bacteriol* 186:4853.

Rosenberg, S.M. and Hastings, P.J. 2004c. Rebuttal: Growth under selection stimulates Lac$^+$ reversion (Roth and Andersson). *J Bacteriol* 186:4862–4863.

Roth, J.R. and Andersson, D.I. 2004a. Rebuttal: Adaptive point mutation (Rosenberg and Hastings). *J Bacteriol* 186:4844.

Roth, J.R. and Andersson, D.I. 2004b. Rebuttal: Adaptive mutation in *Escherichia coli* (Foster). *J Bacteriol* 186:4854.

Roth, J.R. and Andersson, D.I. 2004c. Adaptive mutation: How growth under selection stimulates Lac$^+$ reversion by increasing target copy number. *J Bacteriol* 186:4855–4860.

Schaaper, R.M. 1988. Mechanisms of mutagenesis in the Escherichia coli mutator mutD5: role of DNA mismatch repair. *Proc Natl Acad Sci USA* 85:8126–8130.

Schimke, R.T. 1982. Summary. In: *Gene Amplification*, Schimke, R.T. ed., Cold Spring Harbor, New York. Cold Spring Harbor Laboratory Press, 317–333.

Scheuermann, R., Tam., P.M., Burgers, J., Lu, C., and Echols, H. 1983. Identification of the ε-subunit of *Escherichia coli* DNA polymerase III holoenzyme as the *dnaQ* gene product: a fidelity subunit for DNA replication. *Proc Natl Acad Sci USA* 80:7085–7089.

Scheuermann, R.H. and Echols, H. 1984. A separate editing exonuclease for DNA replication: the ε subunit of *Escherichia coli* polymerase III holoenzyme. *Proc Natl Acad Sci USA* 81:7747–7751.

Shapiro, J.A. 1984. Observations on the formation of clones containing *araB-lacZ* cistron fushions. *Mol Gen Genetx* 194:79–90.

Slupska, M.M., Baikalov, C., Lloyd, R., and Miller, J.H. 1996. Mutator tRNAs are encoded by the *Escherichia coli* mutator genes *mutA* and *mutC*: a novel pathway for mutagenesis. *Proc Natl Acad Sci USA* 93:4380–4385.

Slupska, M.M., King, A.G., Lu L.I., Lin R.H., Mao E.F., Lackey C.A., Chiang, J., Baikalov, C., and Miller J.H. 1998. Examination of the role of DNA polymerase proofreading in the mutator effect of miscoding tRNAs. *J Bacteriol* 180:5712–5717.

Sniegowski, P.D., Gerrish, P.J., and Lenski, R.E. 1997. Evolution of high mutation rates in experimental populations of *E. coli. Nature* 387:703–705.

Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., and Inouye, I. 1966. Frameshift mutations and the genetic code. *Cold Spring Harbor Symp Quant Biol* 31:77–86.

Sutton, M.D. and Walker, G.C. 2001. *Proc Natl Acad Sci USA* 98:8342–8349.

Taddei, F., Matic, I., Godelle, B., and Radman, M. 1997a. To be a mutator, or how pathogenic and commensal bacteria evolve rapidly. *Trends Microbiol* 5:427–428.

Taddei, F., Radman, M., Maynard-Smith J., Toupance, B., Gouyon, P.H., and Godelle, B. 1997b. Role of mutator alleles in adaptive evolution. *Nature* 387:700–702.

Tang, M.J., shen, X., Frank, E.G., O'Donnell, M., Woodgate, R., and Goodman, M.F. 1999. UmuC'D_2C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc Natl Acad Sci USA* 96:8919–8924.

Thilly W.G. 2003. Have environmental mutagens caused oncomutations in people? *Nature Genetics* 34:255–259.

Tlsty, T.D., Albertini, A.M., and Miller, J.H. 1984. Gene amplification in the *lac* region of *E. coli. Cell* 37:217–224.

Trewick, S.C., Henshaw, T.F., Hausinger R.P., Lindahl, T., and Sedgwick, B. Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage. *Nature* 419:174–178.

Turelli, P. and Trono, D. 2005. Editing at the crossroad of innate and adaptive immunity. *Science* 307:1061–1065.

Van Belkum, A., van Leeuwen, W., Scherer, S., and Verbrugh, H. 1999. Occurrence and structure-function relationship of pentameric short sequence repeats in microbial genomes. *Res Microbiol* 150:617–626.

Van Dyk, T.K., Templeton, L.J., Cantera, K.A., Sharpe, P.L., and Sariaslani, F.S. 2004. Characterization of the *Escherichia coli* AaeAB efflux pump: a metabolic relief valve? *J Bacteriol* 186:7196–7204.

Vidmar, J.J. and Cupples, C.G. 1993. MutY repair is mutagenic in mutT strains of *Escherichia coli*. *Can J Microbiol* 39:892–894.

Villesen, P., Aagaard, L., Wiuf, C., and Pedersen, F.S. 2004. Identification of endogenous retrociral reading frames in the human genome. *Retrovirology* 1(1):32–45.

Viswanathan, M., Lacirignola, J.J., Hurley, R.L., and Lovett, S.T. 2000. A novel mutational hotspot in a natural quasipalindrome in *Escherichia coli. J Mol Biol* 302:553–564.

Wagner, J., Gruz, P., Kim, R., Yamada, M., Matsui, K., Fuchs, R.P.P., and Nohmi, T. 1999. The *dinB* gene encodes a novel *E. coli* DNA polymerase pol IV, involved in mutagenesis. *Mol Cell* 4:281–286.

Walker, G.C. 1987. The SOS response of *Escherichia coli*. P. 1346–1357. In: *Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology. Vol. 2.* Neidhardt, F.C., Ingraham, J.L., Low,

K.B., Magasanik, B., Schaechter, M., and Umbarger, H.E., eds. Washington, D. C. American Society for Microbiology, 1346–1347.
Wang, C.L., Harper, R.A., and Wabl, M. 2004. Genome-wide somatic hypermutation. *Proc Natl Acad Sci USA* 101:7352–7356.
Weill, J.C. and Reynaud, C.A. 1992. Early B-cell development in chickens, sheep, and rabbits. *Curr Opin Immunol* 4:177–180.
Wells, R.D. 1996. Molecular basis of genetic instability of triplet repeats. *J Biol Chem* 271:2875–2878.
Whoriskey, S.K., Nghiem, V.-H., Leong, P.-M., Masson, J.-M., and Jeffrey H. Miller. 1987. *Genes Develop.* 1:227–237.
Witkin, E.M. 1974. Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: evidence that ultraviolet mutagensis depends upon an inducible function. *Proc Natl Acad Sci USA* 71:1930–1934.
Witkin, E.M. 1976. Ultraviolet mutagenesis and inducible deoxyribonucleic acid repair in *Escherichia coli*. *Bacteriol Rev* 40:869–707.
Witkin, E.M. and Wermundsen, I.E. 1978. Targeted and untargeted mutagenesis by various inducers of SOS function in *Escherichia coli. Cold Spring Harbor Symp Quant Biol* 48:881–886.
Wood, R.D., Sgouros, M.M., and Lindahl, T. 2001. Human DNA repair genes. *Science* 291:1284–1289.
Woodgate, R. 1999. A plethora of lesion-replicating DNA polymerases. *Genes Develop* 13:2191–2195.
Worth, L., Clark, S., Radman, M., and Modrich, P. 1994. Mismatch Repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. *Proc Natl Acad Sci USA* 91:3238–3241.
Xiong, Y. and Eickbush, T.H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO J* 9:3353–3362.
Yang, H., Wolff, E., Kim, M., Diep, A., and Miller, J. H. 2004. Identification of mutator genes and mutational pathways in *Escherichia coli* using a multicopy cloning approach. *Molec Micriobiol* 53:283–295.
Yoshiyama, K. and Maki, H. 2003. Spontaneous hotspot mutations resistant to mismatch correction in *Escherichia coli*: transcription-dependent mutagenesis involving template switching mechanisms. *J Mol Biol* 327:7–18.