REVIEW ARTICLE

Mutators and hypermutability in bacteria: the *Escherichia coli* paradigm

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Abstract

Mutators (also called hypermutators) are mutants which show higher than normal spontaneous mutation frequencies, ranging from 10–20 fold to 100–1000 fold higher, or sometimes even more, than wild-type cells. Being a mutator is advantageous to the organism when adapting to environmental changes or stressful situations, such as moving from one habitat to another, one host to another, exposure to antibiotics etc. However, this advantage is only a short-term benefit. In the long run, hypermutability leads to a fitness disadvantage due to accumulation of deleterious mutations or antagonistic pleiotropy or both. Contrary to intuitive expectations, hypermutability is commonly encountered in natural bacterial populations, especially among clinical isolates. It is believed to be involved in the emergence of antibiotic resistance and a hindrance to the treatment of infectious diseases. Here, I review the state of knowledge on the common mechanisms of hypermutability such as errors/defects in DNA replication, proof reading, mismatch repair, oxidative DNA damage, mistranslation etc., as well as phenomena associated with these processes, using *Escherichia coli* as a paradigmatic organism.

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Introduction

Mutators, also called hypermutators, are mutant organisms which show higher than normal spontaneous mutation frequencies. The extent of increase may be marginal (10–20 fold) or high (100–1000 fold), or very high (>1000 fold). Quite often one is confronted with the problem of distinguishing between normal bacterial populations and their mutator variants. While the fold increase in the frequency of a standard mutational marker such as rifampicin resistance could be a guiding factor, it fluctuates widely between day-to-day measurements, worker to worker etc. In general, mutator variants of naturally transformable organisms such as species of *Streptococcus, Haemophilus* etc., show ~10-fold increase while non-transformable bacteria such as *Escherichia coli* show 100–1000 fold increase, for any given marker. Mutation frequencies in wild-type cells are usually very low and measurements of the same in wild-type cells are highly variable. These issues have been critically analysed by Hall and Henderson-Begg (2006) who advocate great caution in measuring mutation frequencies, especially low frequencies.

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Acquisition of hypermutability entails a heavy premium in terms of fitness, which is greatly reduced in mutators. On the one hand, mutators can adapt to changing environments and stresses more easily than non-mutators because of their high mutagenic potential; especially if multiple mutations are needed for successful adaptation (Miller *et al*. 1999; also see Denamur and Matic 2006). In this respect, hypermutability is an asset. On the other hand, hypermutability will also be a liability since it would lead to indiscriminate accumulation of unwanted mutations which could be deleterious, or antagonistic pleiotropy whereby presently advantageous mutation(s) might prove harmful, or even lethal , in the long run or under different environmental conditions (Funchain *et al*. 2000; Giraud *et al*. 2001). Issues relating to hypermutability, its fitness costs, evolution of mutation rates etc., have been reviewed extensively (Taddie *et al*. 1997; Sniegowski *et al*. 2000; de Visser 2002; Denamur and Matic 2006). It was intuitively expected that hypermutators would be rare in nature (see Denamur and Matic 2006). However, despite the high fitness costs, hypermutable variants of bacteria are frequently encountered in nature, especially among clinical isolates. There has been a surge of interest in the study of hypermutable bacteria in clinical situations following the initial report of LeClerc *et al*. (1996), who observed that natural isolates of pathogenic strains of *E. coli* and *Salmonella*

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contained an unexpectedly high proportion (1% and above) of mutators. Subsequently, Matic *et al*. (1997) reported that isolates of pathogenic as well as commensal bacteria had a high proportion of mutators. Many reports have appeared before and after that of LeClerc *et al*. (1996), and continue to be on the rise, on the occurrence of hypermutators in several bacterial pathogens and nonpathogens (for references see de Visser 2002; Denamur and Matic 2006). In some cases, such as lung infections in cystic fibrosis patients, the frequency of hypermutators has been found to be extremely high (> 30%; Oliver *et al*. 2000). Presently, hypermutability is implicated in the emergence of antibiotic resistance and also considered to be a major risk factor in the treatment of infectious diseases (Giraud *et al*. 2002; Blazquez 2003; Macia *et al*. 2005; Oliver 2005). Some ideas on how hypermutability could evolve are now beginning to emerge (Sundin and Weigand 2007; Pal *et al*. 2007). The subject of spontaneous mutagenesis, its control and hypermutability have been covered in some earlier reviews (Miller 1996; Horst *et al*. 1999; Maki 2002). In this article, I have attempted to put together some of the old information, for the sake of historical continuity, as well as the more recent developments on the mechanisms of hypermutability from a fundamental, non-clinical point of view in order to present as comprehensive a picture as possible. Since our conceptual understanding of the phenomenon of hypermutability, like other processes and phenomena, has emerged largely, if not exclusively, from studies on *E. coli*, this review will focus on this organism as a paradigmatic model.

Errors in DNA replication and hypermutability

Mutagenesis due to errors in the fidelity of DNA replication is an unavoidable accompaniment of the replication process. In a sense, spontaneous mutagenesis is a 'mere by-product of nature's imperfection: an unavoidable stochastic error' (Radman *et al*. 1999). While DNA replication is very finely regulated to ensure a high degree of fidelity, it is not absolutely error free. In *E. coli* (and also in other organisms) the error frequency is extremely small, of the order of 10^{-10} per nucleotide polymerized. Such a high degree of precision is achieved by three sequential processes during replication; namely, accuracy of base selection, *in situ* proof reading of errors (editing) and post-replicative mismatch repair (MMR). Defects in any one of these processes will result in higher than normal fidelity errors which will manifest as mutations in the subsequent round(s) of replication. All these processes have been extensively studied in many organisms. In *E. coli*, there are five DNA polymerases (DNA polymerases I, II, III, IV and V). A historical account of their discovery as well as the *dramatis personae* involved can be found in the essay by Friedberg (2006). In the following pages, the mechanisms which govern replication fidelity and hypermutability will be discussed.

In *E. coli*, DNA replication is carried out mainly by the DNA polymerase III holoenzyme complex, which consists of 17 protein components of 10 types (detailed information on these entities can be found in standard text books and will not be repeated here unless relevant). The principal component of the replication machinery is the ($\alpha\varepsilon\theta$) complex, called the core polymerase. Of these, α is the catalytic (polymerization) subunit (encoded by *dnaE*), ε is the proof reading subunit (encoded by *dnaQ*) and θ (encoded by *holE*) is the stabilizer of the $\alpha\varepsilon$ complex. Many point mutations in *dnaE*, conferring temperature sensitive replication defects and mutator phenotypes at the permissive temperature have been isolated (for references, see Vandewiele *et al*. 2002). Sequence analyses of some of these showed that the lesions are not localized to any particular region of the gene, such as the active site, but are randomly spread across the gene (Vandewiele *et al*. 2002). While the extent of hypermutability in most of the *dnaE* mutants is only marginal (~10–20 fold), one mutant, *dnaE 173*, bearing a glu 612 lys substitution, is a very strong hypermutator (> 3000 fold; Maki *et al*. 1991; Mo *et al*. 1991). Vandewiele *et al*. (2002) made the interesting observation that the mutator phenotype of the *dnaE* mutants they studied strongly depended on the presence of active DNA polymerase V (a member of the Y family of DNA polymerases; see below) since deletion of the genes encoding it (*umuD,C*) decreased, but did not abolish, mutator activity (these experiments were done in a strain in which *umuD,C* is constitutively expressed whereas in wild-type strains DNA polymerase V is hardly detectable since *umuD,C* expression needs SOS induction; see below). However, even in a Δ *umuD,C* genetic background, the *dnaE* mutants showed modest mutator activity (5–20 fold; Vandewiele *et al*. 2002) which is perhaps a reflection of their true mutagenic potential. Vandewiele *et al*. (2002) suggested that the mutator activity of most of the *dnaE* mutants could be attributed to the reduced ability of the mutant DNA polymerase III to resist competition by other DNA polymerases, especially DNA polymerase V (if present), to take over replication. The very high mutator activity of the *dnaE 173* mutant (see above) seems to be due to impaired α–ε subunit interaction, resulting in defects in proof reading, and consequent hypermutagenesis (see below; Maki *et al*. 1991; Mo *et al*. 1991).

While mutator mutations increase mutation frequencies, antimutator mutations are those which decrease the same. Many antimutator mutations mapping in *dnaE* have been isolated and characterized in Schaaper's laboratory (reviewed by Schaaper 1998); see also Vandewiele *et al*. 2002). While reviewing the work in his laboratory on antimutators of phage T4 and *E. coli*, Schaaper (1998) has discussed some of the theoretical issues involved in the relative ease of isolation of mutators *vis-a-vis* antimutators. Some reservations on the occurrence of antimutator mutations have also been raised earlier by Drake (1993). Nevertheless, antimutators have been isolated in phage T4 as well as *E. coli* (reviewed

by Schaaper 1998). Unlike the DNA replication apparatus of *E. coli* which is a multi-protein complex, the DNA polymerase of phage T4 (encoded by the T4 gene 43) is a single polypeptide carrying out both polymerisation and *in situ* proof reading (carried out by an associated exonuclease activity), each acting in opposite directions, the former acting in the $5' \rightarrow 3'$ direction and the latter in the $3' \rightarrow 5'$ direction. Speyer (1965) was the first to describe a mutant T4 DNA polymerase with mutator activity. Analysis of a large collection of temperature sensitive gene 43 mutants of phage T4 by Drake *et al*. (1969) revealed the presence of both mutators and antimutators in the collection. A detailed study of wild-type, mutator and antimutator T4 DNA polymerases by Muzycka *et al*. (1972) showed that the ratios of exonuclease / polymerase activities were in the order: antimutators > wild type > mutators, suggesting that the fidelity of replication is determined not only by the accuracy of base selection but also by proof reading (for a detailed discussion, see Schaaper 1998).

The antimutator alleles of *dnaE* referred to above were isolated by Schaaper and his group using a novel strategy; namely, as suppressors of the mutator phenotype of mismatch repair defective *mutL* mutants (see below). All the isolates had single amino acid substitutions in *dnaE* and reduced spontaneous mutation frequencies by 3–30 fold. The antimutator activity was more pronounced in reducing the frequencies of transversions than transitions, suggesting that the former could be the result of base misincorporation errors while the latter could be due to DNA damage-related mispairing errors which could be corrected by the mismatch repair processes (see below). Interestingly, DNA polymerase III from the wild type and *dnaE* antimutator mutants did not differ in their base insertion fidelity. Moreover, the antimutator alleles were effective in other genetic backgrounds (such as *mutD5* and *dnaQ926*; see below) which are defective in proof reading (see Schaaper 1998). Therefore, their antimutator phenotype seems to be due to more efficient error correction than greater accuracy of base selection (Fijalkowska and Schaaper 1993, 1995).

Defective proof reading and hypermutability

With the exception of *dnaE173*, all the mutator alleles of *dnaE* studied so far show weak to moderate mutator effects. *E. coli* mutants exhibiting very strong mutator activities (several hundred fold higher than normal) were isolated long ago by Degnen and Cox (1974) and Horiuchi *et al*. (1978) and characterized very well by several workers (for references, see Taft-Benz and Schaaper 1998). These mutations mapped at a locus which has been called *mutD* by some workers and *dnaQ* by others. The product of the *mutD/dnaQ* locus was identified as the ε subunit of DNA polymerase III and shown to possess a $3' \rightarrow 5'$ exonuclease activity (Scheurmann *et al*. 1983) which removes mismatched nucleotides at the replication fork (Scheurmann and Echols 1984). Therefore, it could be expected that defects in the exonuclease activity would allow the mismatches to persist and manifest as mutations subsequently. In agreement with this notion, a clear correlation between exonuclease deficiency and mutator activity was demonstrated (Echols *et al*. 1983; Di Francesco *et al*. 1984). The first *mutD/dnaQ* mutants to be isolated were *mutD5* and *dnaQ49* (Degnen and Cox 1974; Horiuchi *et al*. 1978). Subsequently, several mutants in the same locus were isolated and characterized by Taft-Benz and Schaaper (1998). Some of these were moderate mutators (6–20 fold higher than normal), while some others were strong to very strong mutators (700–8000 fold higher). Complementation analysis using plasmid-borne $dnaQ^+$ allele showed that they could be grouped into three classes: dominant, recessive and partially dominant. The dominance/recessiveness of these mutants might be a reflection of their catalytic efficiency *vis-a-vis* ability to complex with the α subunit of the polymerase III complex (Taft-Benz and Schaaper 1998). The above authors have also discussed at length the significance of the motifs in which the mutations map in relation to their catalytic activity and structural relevance.

The *mutD/dnaQ* mutants are impaired in viability depending upon the strength of their mutator effects. For instance, the *mutD5* mutant is poorly viable. The *dnaQ926* mutant, constructed by site-specific mutagenesis by changing the two conserved amino-acid residues (asp12 and glu14 to ala12 and ala14, respectively) conferred a strong and dominant mutator phenotype, stronger than *mutD5*, when carried on a plasmid. However, when present on the chromosome, the *dnaQ926* mutation completely abolished viability unless the cell also carried antimutator *dnaE* mutations such as *dnaE911, dna E915, dnaE925* etc. (Fijalkowska and Schaaper 1996). This phenomenon has been called 'error catastrophe' in which the extent of mutagenesis is so much that the accumulation of deleterious mutations results in loss of viability. Although the mutator phenotype could be correlated with deficiency of exonuclease activity, the indirect role of the α subunit should also be taken into account. It has been shown that the exonuclease activity of the ε protein is strongly stimulated by the α protein *in vitro* (Maki and Kornberg 1987). As pointed out earlier, the strong mutator activity of the *dnaE 173* mutant is brought about through its effect on proof reading (Maki *et al*. 1991; Mo *et al*. 1991).

Role of the θ subunit of DNA polymerase III in hypermutability

Besides the α and ε subunits involved in replication and proof reading functions, respectively, the DNA polymerase III core complex has a third subunit called θ, which is the product of the *holE* gene (Carter *et al*. 1993; Studwell-Vaughan and O'Donnell 1993). The θ subunit does not bind to the α but binds to the ε subunit, stimulating its exonuclease activity 2–3 fold (Studwell-Vaughan and O'Donnell 1993). The role

of the θ subunit in replication fidelity has come to light recently when Taft-Benz and Schaaper (2004) reported that it could stabilize the α–ε complex. The mutator activity of the *dnaQ49* (ϵ mutant) is temperature dependent. At a low temperature (25°C) it is only moderate (10-fold) but at 30^0C and 37^0C it is very strong (100 and 1000 fold, respectively). The lesion, namely, a V96G amino-acid substitution, renders the ε protein of the *dnaQ49* mutant heat labile and leads to loss of binding to the α subunit at high temperature and consequent failure of proof reading (for references, see Taft-Benz and Schaaper 2004). A Δ*holE, dnaQ49* double mutant is viable but shows a very strong mutator phenotype even at 25°C (Taft-Benz and Schaaper 2004) i.e., even at 25^0C it shows the phenotype which a *holE*$^+$ *dnaQ49* mutant would at 37°C. Similarly, the mutator effects of a few other *dnaQ* alleles (*dnaQ920, 923, 924* and *928*) are also enhanced in *holE* deletion strains. Based on these results, Taft-Benz and Schaaper (2004) suggested that the role of the θ subunit could be to stabilize the α-ε complex. In a sense, the θ subunit functions as an antimutator since its presence decreases mutation frequency and its absence increases the same. Interestingly, the θ subunit of *E. coli* can be effectively replaced by the *hot* gene product of phage P1 (Chikova and Schaaper 2005). Like θ, the P1 *hot* gene product also exerts an antimutator effect on the *dnaQ* mutants mentioned above (except *dnaQ928*). In a subsequent report, Chikova and Schaaper (2006) showed that the P1 *hot* gene product enhanced the mutator activity of another *dnaQ* allele (*dnaQ930*) i.e., it can also act as a mutator in some genetic backgrounds. Interestingly, the mutator effect of the P1 *hot* gene product was observed in a *dnaQ*$^+$ strain also, suggesting that the θ and Hot proteins compete with each other for binding to the α–ε complex. Taken together, these reports show that the interaction of θ or Hot protein with ε can either augment or weaken the efficiency of proof reading, depending on the *dnaQ* allele. Although *holE* is nonessential for viability, its product seems to play an important role in replication fidelity.

The role of the τ subunit of DNA polymerase III in hypermutability

As already noted, the core polymerase complex (αεθ) is the main entity of replication and proof reading. Several accessory proteins also participate in the process to ensure processivity and simultaneous replication of leading and lagging strands (reviewed by Mc Henry 2003; O'Donnell 2006). These comprise of the τ subunit which tethers the core complex to the DNA, and the Dna X complex ($\tau_2\gamma\delta\delta'\chi\Phi$; see Mc Henry 2003; O'Donnell 2006). The τ and γ polypeptides are encoded by a single gene, *dnaX*. While τ is the full length product (71 kDa) of *dnaX*, γ (47.5 kDa) arises from premature translation termination of the *dnaX* transcript (Mc Henry 2003). Two *dnaX* mutants (*dnaX36* and *dnaX2016*) have been isolated earlier by Henson *et al*. (1979) and six more (*dnaX983, 985–989*) were isolated recently by Pham *et al*. (2006). The τ and γ proteins have three domains in common (domains I, II and III) while τ has two unique domains (IV and V). All the *dnaX* mutations referred to above are located in domain V of τ. Pham *et al*. (2006) have reported some unusual mutator properties of the *dnaX* mutants in that they specifically enhance the frequency of transversions (GC → TA and AT → TA, 65 and 32-fold, respectively) more than transitions (GC → AT and AT → GC, 2–4 fold). In addition, they also moderately enhance the frequency of −1 frameshifts in non-run sequences. These characteristics are believed to be unique among *E. coli* mutators (Pham *et al*. 2006). Pham *et al*. (2006) proposed a model to explain the mutator phenotype of the *dnaX36* mutant, invoking a role for the τ subunit in sensing misincorporation errors (see below). In a subsequent report, Gawel *et al*. (2008) extended the analysis of the *dnaX36* mutant, also probing the role of DNA polymerases I, II, IV and V and other gene products in preventing or enhancing its mutator phenotype. The model originally proposed by Pham *et al*. (2006) and subsequently refined by Gawel *et al*. (2008) is as follows. When the DNA polymerase III holoenzyme (HE) stalls at a base mispair as a non-productive complex (neither proofread nor extended), the resolution of the complex is proposed to involve the α–τ subunit interaction. The simplest option, namely, switching over to the proof reading mode is proposed to be impaired in the *dnaX36* mutant. One of the other options is the forced extension of replication beyond the mismatch resulting in fixation of the mutation, an event which is independent of the other DNA polymerases. Yet another option is dissociation of the HE from the replication fork, thereby providing an opportunity for other polymerases (pol II or pol IV) or pol III itself (in the proof reading mode) to resume replication. Pol IV, being an error-prone enzyme will generate mutations if it succeeds while any one of the other two will correct the error if it succeeds (for more detailed information see Gawel *et al*. 2008).

Hypermutability due to defective mismatch repair

When a mismatched base at the replication fork escapes *in situ* correction by the proof reading exonuclease, and replication has advanced further downstream, the mismatch repair (MMR) system comes into play to correct the mismatch. In exonucleolytic proof reading, the parent and progeny strands could be easily distinguished since the latter is only partially replicated. However, in mismatch repair the two strands cannot be distinguished since replication might have advanced beyond the mismatch. This poses a problem for the repair machinery, namely, it is the wrong base on the progeny strand that should be corrected with respect to the right one on the parent strand and not *vice versa*. In *E. coli* a way to get around this predicament exists. The adenine residues in GATC sequences of *E. coli* DNA are methylated at N-6, by an enzyme (DNA adenine methylase) encoded by the *dam*

gene. Since GATC methylation is a post-replicative event, newly replicated DNA exists in a hemi-methylated state for short periods of time, with the parent strand bearing methyl groups and the progeny strand lacking them (yet to be methylated). The repair machinery exploits this time interval to do its job. If mismatch correction fails to occur during this time interval and the progeny strand also gets methylated meanwhile, the repair process slows down considerably and might even fail. This would result in fixation of the mutation instead of repair of the mismatch. Mutations in the *dam* gene result in under methylation of the parent as well as the progeny strands and consequent reduction / loss of the parent-progeny distinction and strand bias in mismatch repair. Therefore, *dam* mutants are (moderate) hypermutators (Marinus and Morris 1974). Interestingly, overexpression of wild-type *dam* gene also results in moderate hypermutability because of rapid, post-replicative methylation of progeny strand and reduction in the time available for mismatch repair (Herman and Modrich 1981). The process of mismatch repair has been reviewed extensively (Schofield and Hsieh 2003; Kunkel and Erie 2005; Iyer *et al*. 2006; Jiricny 2006; see also Friedberg 2006).

Mismatch repair deficiency is by far the most common cause of hypermutability in clinical isolates. The key players in mismatch repair processes are the products of *mutS, mutL, mutH* and *uvrD* genes. Several other proteins such as ExoI, ExoVII and ExoX nucleases, RecJ protein, DNA polymerase III, DNA ligase etc. are also involved. Mismatch repair is a multi-step process, initiated by the binding of the MutS dimer to the mismatch followed by ATP-dependent binding of the MutL dimer. The mismatch-$(MutS)_2$-$(MutL)_2$ ternary complex activates the latent endonuclease activity of the MutH protein which nicks the unmethylated strand of the hemi-methylated DNA, opposite the methylated GATC, either 5′ or 3′ to the mismatch. The nicking occurs 5′ to the G. The product of *uvrD* (helicase II) unwinds the DNA at the nicked site, followed by the degradation of the DNA between the nick and the mispair in the 5′ → 3′ or 3′ → 5′ direction depending upon the location of the nick. The ExoVII or RecJ proteins are implicated in the former and ExoI or ExoVII or ExoX in the latter. This results in a gap which gets filled in by repair synthesis by DNA polymerase III, followed by ligation. Details of this process can be found in the reviews cited above. Hypermutability resulting from MMR deficiency is very strong (100–1000 fold). Besides mismatch repair, GATC methylation effected by the *dam* methylase is involved in several other cellular processes (reviewed by Lobner-Oleson *et al*. 2005).

In addition to their role in mismatch repair the MutS, MutL, MutH and UvrD proteins also function as inhibitors of what is known as homeologous or quasi-homologous recombination, i.e. recombination between largely homologous, but not identical, sequences. It has been known for long that conjugational recombination between *E. coli* donors and *Salmonella* recipients is very poor although their chromosomes are ~80% homologous. However, if the recipient is MMR deficient then the yield of recombinants increases 100–1000 fold (Rayssiguier *et al*. 1989). Stimulation of interspecies recombination is very high in *mutS* and *mutL* recipients and somewhat less in *mutH* and *uvrD* strains. The yield of transductional recombinants also was observed to be stimulated many fold in heterospecific crosses (*E. coli* donors × *S. typhimurium* recipients) if the recipients were *mutS* or *mutL* (Rayssiguier *et al*. 1989). Funchain *et al*. (2001) have used the promotion of homeologous recombination to enrich mutators in a population. They reported that after two cycles of heterospecific conjugational crosses between a *Salmonella* donor and an *E. coli* recipient more than 95% of the selected recombinants were MMR deficient and hence mutators. Similarly the occurrence of large duplications of genomic segments was higher in MMR deficient backgrounds (Petit *et al*. 1991). This is believed to be due to inhibition of generation of such duplications by intrachromosomal recombination in wild-type (mismatch repair proficient) cells and its alleviation in mismatch repair deficient mutants. The two characteristic phenotypes of MMR deficient mutants; namely, hypermutability and promotion of homeologous recombination are biologically very significant. As pointed out earlier, hypermutability is advantageous to the cell in adapting to new/changing/stressful environments. Once adaptation is achieved, the continued presence of mutant MMR genes (through hitch-hiking) is indeed a liability. It was expected that reacquisition of wild-type MMR genes either through reversion (an unlikely possibility; see de Visser 2002) or through horizontal gene transfer from disparate donors followed by homeologous recombination would not only enable the cells to retain the advantageous mutation but also save them from possible extinction due to hypermutability. In agreement with this notion, many workers have found that MMR genes show considerable sequence mosaicism derived from diverse lineages (Denamur *et al*. 2000; Brown *et al*. 2001). The biological significance and possible applications of interspecies recombination, stimulated by *mutS–mutL* mutations, have been discussed at length by Rayssiguier *et al*. (1989).

Recently, Hong *et al*. (2005) have isolated temperature sensitive *mutS* mutants. One of these (*mutS60*, a GC → AT transition resulting in ala 134 val substitution in the MutS protein) shows some interesting properties. It shows near normal mutation frequencies at 30°C but behaves as a *mutS* null mutant (hypermutator) at 43°C. At an intermediate temperature (37°C) the mutant is not a hypermutator (phenotypically $mutS^+$) but it does not inhibit homeologous recombination as a genuine $mutS^+$ strain would. While it allows homeologous recombination to occur, it is not as effective as a *mutS* null mutant either in this regard (Hong *et al*. 2005). The low mutability of some weak *mutD* (proof reading defective) mutants, as well as a *ndk* (nucleoside diphosphate kinase) mutant, is enhanced several fold in the *mutS60* mutant at 37°C (Hong *et al*. 2005).

Hypermutability due to defects in handling oxidative DNA damages

Reactive oxygen species (ROS) such as superoxides, hydroxyl radicals, hydrogen peroxide etc. are the by-products of normal cellular metabolism and also arise due to exposure to ionizing radiations, carcinogens, xenobiotics etc. (reviewed by Bjelland and Seaberg 2003; Imlay 2003). As a first line of defence against these harmful agents, cells deploy enzymes such as superoxide dismutase and catalase which destroy them. Nevertheless, oxidative damage is a serious threat to DNA and other cellular macromolecules. Several oxidatively damaged purine and pyrimidine bases are known. Among these, an oxidized guanine molecule, namely, 8-oxo, 7–8 dihydro guanine (8-oxodG) which is a very stable compound, has received extensive attention because of its high mutagenic potential. In the *syn* conformation, 8-oxodG mimics T in its base pairing capacity and can pair with A (*anti*) while in the *anti* conformation it can pair with C (*syn*). Structural studies have shown that 8-oxodG (*syn*): A (*anti*) base pairs mimic normal T : A base pairs and could be replicated without difficulty. However, the 8-oxoG (*anti*): C (*syn*) basepairs induce distortions in the template as well as the polymerase (Shibutani *et al*. 1991; Hsu *et al*. 2004). The 8-oxo dG:dA base pair is a pre-mutational lesion which could result in G : C → T : A transversion in the next round of replication. The mutagenic potential of 8-oxodG lesions is comparable to *mutD/ dnaQ* (proof reading defective) mutations and about 10-fold stronger than *mutS/mutL* (mismatch repair defective) mutations. There are two ways by which 8-oxodG lesions can arise in DNA. Free dGTP in the dNTP pool can be oxidized to 8-oxo dGTP and then get incorporated into replicating DNA, opposite template C or A. Alternatively, G residues in DNA can be oxidized to 8-oxodG. Depending upon the mode of generating the 8-oxodG lesion, AT → CG or GC → TA transversions will result. Avoiding and/or repairing 8-oxodG lesions have been well characterized in *E. coli* and other prokaryotes as well as eukaryotes, including humans. Briefly, there are three mechanisms, one to preempt 8-oxo dGTP getting incorporated into DNA, and the other two to repair the damage if it succeeds in doing so. In *E. coli*, these mechanisms are under the control of the products encoded by *mutT, mutM* and *mutY* genes (Michaels and Miller 1992; Grollman and Moriya 1993; Tajiri *et al*. 1995; Fowler and Schaaper 1997; Fowler *et al*. 2003). Cartoons depicting mutgenesis by 8-oxodG can be found in the above reviews. Some salient features of these processes are described below.

The product of the *mutT* gene is a nucleoside triphosphatase which preferentially hydrolyses 8-oxo dGTP to 8-oxo dGMP and PPi. This reaction is an error avoidance mechanism to prevent 8-oxo dGTP from getting into the DNA. The MutT protein is a member of the family of proteins called the nudix enzymes (Bessman *et al*. 1996). Nudix enzymes are hydrolases which act on a nucleoside (nu) diphosphate (di) linked to some other moiety (x). Some examples of nudix enzymes are those acting on ADP-ribose, GDP-mannose etc., their biological function is believed to be in house cleaning and sanitizing cells from potentially harmful metabolites (Bessman *et al*. 1996). The *mutT* mutants show very high frequencies (3000-fold or higher) of A : T → C : G transversions. This suggests that 8-oxodG not only gets into DNA without much difficulty but also that the resulting mispairs are not effectively repaired by the proof reading and/or mismatch repair systems. For more and detailed information on the role of *mutT* in replication fidelity, see the review by Fowler and Schaaper (1997).

While the MutT protein restricts the availability of 8-oxo dGTP for incorporation into DNA, the products of two other loci: *mutM* and *mutY*, come into play after incorporation, if any, has occurred. The MutM and MutY proteins are glycosylases which remove damaged or misincorporated bases to initiate nucleotide excision repair (NER). The MutM glycosylase removes 8-oxoG paired with C and the MutY glycosylase removes A (mis) paired with 8-oxoG. Strains with defects in *mutM* and *mutY* are strong mutators giving rise to high frequencies of GC → TA transversions (reviewed by Fowler *et al*. 2003). In *mutM–mutY* double mutants hypermutability is very strong (Fowler *et al*. 2003). Structural biological studies on how the MutM and MutT glycosylases recognize their respective substrates against an enormous abundance of normal base pairs have been reviewed recently (David *et al*. 2007).

2-Oxo-dATP is yet another abnormal nucleotide resulting from oxidative damage. Yamada *et al*. (2006) showed that DNA polymerase IV (see below) could incorporate 2-oxo-dATP opposite template dG or dT and 8-oxo-dGTP exclusively opposite dA *in vitro*. Such misincorporations *in vivo* could result in G : C → T : A and A : T → C : G transversions. An *E. coli* strain (QC 1736) which lacks both the genes for superoxide dismutases (*sodA* and *sodB*) as well as the gene *fur* (a negative regulator of iron uptake, displays high mutation rates for such transversions (Yamada *et al*. 2006)). This effect is alleviated in mutants lacking DNA polymerase IV or V. Similarly over expression of *mutT* (or its human homologue MTH1) which could hydrolyse 2-oxo-dATP in addition to 8-oxo-dGTP also reduces the severity of the mutator phenotype of the *sodA-sodB-fur* mutant (see Yamada *et al*. 2006, for references). Bridges (1996) reported that mutational reversion of some auxotrophic mutations is greatly elevated in *mutT, mutM, mutL* and *mutT mutL* genetic backgrounds under conditions of starvation for the required nutrients (adaptive mutagenesis). These results suggested that there could be significant levels of DNA turnover even in apparently non-growing cells.

Roles of other DNA polymerases in hypermutability

As mentioned earlier, there are five DNA polymerases in *E. coli* (DNA polymerases I to V). The foregoing discussion

concentrated almost exclusively on pol III because it is the most important among all the five. While the genes encoding polymerases I, II, IV and V could be deleted and hence are nonessential for cell viability, *dnaE* which encodes the a subunit of DNA polymerase III is an essential gene; only conditional lethal (temperature sensitive) mutants are known in *dnaE*. In the following section, the roles of the other DNA polymerases in hypermutability will be discussed. DNA polymerases IV and V, encoded by the *dinB* and *umuD,C* genes, respectively, belong to the Y family (see Friedberg 2006) and form part of the SOS regulon. It has been estimated that the normal *E. coli* cells have ~250 molecules of pol IV / cell and an undetectable number of pol V molecules (<15 / cell). Although the level of pol IV is ~8-fold higher than pol III, the main replicative polymerase, *dinB* deletion mutants do not display any crippling phenotype(s) and are fully viable. Cells lacking pol V are also viable but are nonmutable by UV. Several aspects of the biology of these enzymes and the genes encoding them have been reviewed extensively (Goodman 2002; Fuchs *et al*. 2004; Nohmi 2006; Schlacher *et al*. 2006; Yang and Woodgate 2007). The role of pol IV in replication fidelity and hypermutagenesis has been studied extensively by Kuban *et al*. (2005, and references cited therein) and also reviewed by Matic (2008). There is a general consensus that pol IV, when present at basal levels (250 molecules/cell), has no significant effect on chromosomal mutations in growing cells, although Strauss *et al*. (2000) have reported that 40%–60% of replication errors in growing cells could be attributed to the action of pol IV. It influences mutagenesis of F' episomes moderately (see Kuban *et al*. (2005) for references). However, mutagenic events in nongrowing cells (adaptive mutagenesis) and those triggered by DNA damage are strongly dependent on pol IV (see Kuban *et al*. 2005)

As pointed out earlier, there are < 15 molecules of pol V in a normal cell. Pol V is a complex entity consisting of a homodimer of cleaved UmuD protein (UmuD′) and one molecule of UmuC, i.e., UmuD′$_2$C (see Friedberg *et al*. 2006). The cleavage of UmuD requires RecA protease activity (RecA*) arising from DNA damage followed by SOS induction or mutations in *recA* (such as *recA441, recA730* etc.), which bypass the need for DNA damage and lead to constitutive expression of SOS genes (see Friedberg *et al*. 2006). Kuban *et al*. (2006) have reported a detailed analysis of SOS mutator activity in *recA730* mutants. They showed that it depends not only on pol V but also on pol IV although the requirement for the former is more critical. The pol V/pol IV mediated (SOS) mutator activity shows a preference for the lagging strand and results in transversion errors. It was also suggested that the pol IV–pol V interaction is cooperative rather than additive. For a detailed discussion and references see Kuban *et al*. (2006). It should be noted that defects/loss of pol IV and pol V result in reduction of mutation frequencies (antimutator effects) whereas defects in other genes (*dnaQ, mutS, mutL* etc.), enhance the same and result in a hypermutator phenotype (see the earlier sections).

DNA polymerase I was the first DNA polymerase to be discovered (see Friedberg 2006). It functions mainly in filling single strand gaps in duplex DNA arising during lagging strand replication and also during nucleotide excision repair (NER; see Friedberg *et al*. 2006 for details). Pol I, encoded by *polA*, is a single polypeptide (103 kDa) which has three enzymatic activities: the polymerase, $3' \rightarrow 5'$ exonuclease and $5' \rightarrow 3'$ exonuclease. Structural damages suffered by DNA, either spontaneously, or due to exposure to DNA damaging agents, are repaired by the nucleotide excision repair (NER) pathway initiated by the products *uvrA, uvrB , uvrC* and *uvrD* genes , generating short single stranded gaps (about 13–14 bases long) in duplex DNA. These gaps are filled in by repair synthesis by pol I. Therefore, it could be expected that defects in or loss of NER genes will have mutator effects. Surprisingly, Hasegawa *et al*. (2008) observed exactly the opposite, i.e., mutants deleted for *uvrA, uvrB* and *uvrC*, displayed a 40%–80% reduction in spontaneous mutation frequency. While mutations in the NER genes have antimutator effects, their overexpression leads to spontaneous SOS induction and mutator effects (Hasegawa *et al*. 2008). Cells with a $3' \rightarrow 5'$ exonuclease-deficient pol I were found to be hypermutators; the mutator effect in such cells was also reduced by deletion of NER genes. These results show that pol I and the NER genes are somehow involved in spontaneous mutability. Hasegawa *et al*. (2008) have suggested that even normal cells suffer (unwarranted) nucleotide excision repair, and error-prone gap filling by pol I generates mutations, exacerbated if the pol I happens to be $3' \rightarrow 5'$ exonuclease defective. It is not clear at the moment as to what exactly is/are the target(s) for such unprovoked excision repair. It is worth noting that DNA repair excision nucleases have been shown to act on undamaged DNA (gratuitous repair) and implicated in spontaneous mutagenesis (Branum 2001).

Like DNA polymerases IV and V, DNA polymerase II (pol II , encoded by *polB*) is also under SOS control. Uninduced cells have ~50 molecules of pol II/cell which increase seven-fold upon SOS induction. Many biological functions such as replication of UV and/or oxidatively damaged DNA, restart of stalled DNA replication in UV irradiated cells, stationary phase mutagenesis and many others have been attributed to pol II (for references, see Al Mamun 2007). Pol II is a single polypeptide (90 kDa) with an associated $3' \rightarrow 5'$ exonuclease proof reading activity, the only one among the three SOS-controlled DNA polymerases to possess it. Using a proof reading defective *mutD5* mutant (see above), also deleted for pol II and / or poll IV and / or pol V, Nowosielska *et al*. (2004) have examined mutgenesis during exponential growth as well as stationary phase, using the reversion of an ochre mutation in *argE* (*argE3*) to arginine prototrophy as a marker. Since *mutD5* strains are chronically SOS induced (for references, see Nowosielska *et al*. 2004), all the three SOS controlled polymerases are expected to be present at their respective induced levels in *mutD5* cells. The important

observation they made was that during exponential growth, *mutD5* Δ *polB* mutants were strong mutators while *mutD5* Δ *dinB* or *mutD5* Δ *umuD,C* were relatively less strong, if not weak mutators. In the stationary phase, however, the mutator activity was in the order: Δ *dinB* > Δ *umuD, C* > Δ *polB*. The enhancement of the hypermutability of the *mutD5* allele by Δ *polB* (1.6-fold) was taken to indicate that pol II could compensate to some extent the proof reading defect of the *mutD5* mutant. Al Mamun (2007) reported that pol II at its basal or moderately elevated (~6 fold) levels does not contribute significantly to spontaneous mutagenesis. However when the level is elevated >10 fold spontaneous frequency is increased more than 100 fold. This effect was independent of SOS induction , pol IV, pol V, *mutS, uvr A* etc., When *polB* carrying a mutation in its exonuclease motif (a D156 A change) was overexpressed, the spontaneous mutation frequency increased 20000 fold, i.e., 200 fold higher than over expression of *polB*$^+$. Mutations in a short stretch of amino acids at the C-terminal end of pol II (conserved in pol II, pol IV and pol V) abolish the mutator activity of pol II upon over expression. How a high fidelity DNA polymerase such as pol II could behave like a mutator when over expressed and the physiological relevance of this effect, if any, are matters for speculation; Al Mamun (2007) has considered and discussed several possibilities. For more information on pol II, pol IV and pol V see the reviews cited earlier.

Hypermutability due to translational errors

Michaels *et al*. (1990) identified two mutator loci in *E.coli*: namely, *mutA* and *mutC*, mapping at 95 and 43 min, respectively. The frequency of transversions, especially A : T → T : A and G : C → T : A were enhanced many fold in these strains. Subsequently, Slupska *et al*. (1996) identified the *mutA* and *mutC* genes to be the ones coding for glycyl tRNA. In the *mutA* mutant, one of the three glycyl tRNA genes (*glyV*, located at 95 min) is mutated while in *mutC* the single copy of the same gene (*glyW*, located at 43 min) is mutated. In both the mutants, the lesion is located at the anticodon part of the gene such that the mutant glycyl tRNAs recognize aspartic acid codons (GAU or GAC) instead of glycine codons (GGU or GGC). This would result in translational errors such that aspartic acid residues in proteins could occasionally be replaced by glycine residues. In *mutA-mutC* double mutants the mutation frequency was 2–3 fold higher relative to the single mutants. Slupska *et al*. (1996) suggested that mistranslation in the *mutA/mutC* mutants caused by occasional replacement of aspartic acid residues by glycine in some critical determinant of replication fidelity such as the ε subunit could corrupt its biological activity and give rise to hypermutagenesis, i.e., a *mutD/dnaQ* phenotype. Since the *mutA/mutC* mutator activity is only of the order of 1% of a strong mutator like *mutD5*, it is possible that only ~1% of the ε subunits are corrupted. Subsequently, Slupska *et al*. (1998) constructed mutants in which each of the 16 aspartic acid residues in the ε protein was replaced, one at a time, by glycine and examined their mutator phenotypes. It turned out that three of the constructs (D12G, D103G and D167G) were very strong mutators and one (D129 G) was a relatively less strong mutator. Slupska *et al*. (1998) also isolated a mutant in which *glyU* was mutated to read histidine codons. They examined the consequences of replacing each of the seven histidine residues in ε protein by glycine. Two replacements (H98G and H16G) yielded very strong mutators. The implications of these findings are discussed in detail by Slupska *et al*. (1998).

In a series of elegant papers, Humayun and co-workers have reported extensively on mutagenesis caused by translational errors, a phenomenon which they have named 'translation stress induced mutagenesis' (TSM: for references see Humayun 1998; Balashov and Humayun 2003; Al Mamun *et al*. 2006). It is well known from the classical work of Gorini and co-workers that the products of two genes which encode proteins of the 30 S ribosomal subunits control the accuracy of translation. One of them is *rpsL* (earlier called *strA*) encoding the S12 protein and the other is *rpsD* (earlier called *ram*) encoding the S4 protein. Mutations in the former give rise to streptomycin resistance and lead to increased translation accuracy while mutations in the latter show relaxed translation accuracy and display what has been called the ribosomal ambiguity (Ram) phenotype, characterized by a high degree of translational errors (reviewed by Gorini 1969). Balashov and Humayun (2003) observed that an *rpsD14* mutant displayed high rates of reversion of a *lacZ* mutant to *lacZ*$^+$ (A : T → T : A). This effect was unaffected by *recA* and *lexA* mutations, i.e., not SOS-dependent but was countered by an *rpsL* mutation (*rpsL1408*) in the absence of streptomycin but not in its presence. The *rpsL1408* mutation by itself showed a streptomycin-dependent mutator effect. When both *rpsD14* and *rpsL1408* were present, the streptomycin-dependent mutator phenotype due to the latter prevailed (Balashov and Humayun 2003). These results suggested that generalized mistranslation (occurring in the *rpsD/rpsL* mutants, as against specific mistranslation in *mutA/mutC* mutants), is also highly mutagenic. Balashov and Humayun (2003) favour the hypothesis that generalized mistranslation results in (unspecified) alterations in pol III and consequent hypermutability. Humayun and co-workers have also probed in greater detail the mutator properties of *mutA/mutC* mutants. These mutants display another interesting phenotype: an SOS-independent, mutagenic repair of lesions created by 3, N^4-ethenocytosine, which forms noninstructional, bulky adducts on the phage M13 single stranded DNA. In wild-type cells this repair process is *recA* independent but needs induction by UV. However, in *mutA/mutC* cells it is UV-independent (constitutive) but *recA* dependent, as are the mutagenic events (reviewed by Humayun 1998). In a recent paper, Al Mamun *et al*. (2006) showed that in *mutA* cells mutagenesis of phage T4 genome is enhanced 4–

10 fold and the proportion of cells which suffer spontaneous induction of a (defective) prophage is enhanced 7–10 fold. Since T4 DNA replication is completely independent of the host's replication machinery, they proposed that occasional mistranslation in *mutA* cells corrupts the replicative polymerase rather than the accessory elements, as was proposed earlier by Slupska *et al*. (1996, 1998). However corruption of the ε subunit by mistranslation cannot be totally ruled out. The *recA* dependence for mutagenesis and prophage induction in *mutA* cells suggest that some cells in the population suffer episodic replication fork collapse and need recombination functions for recovery (Al Mamun *et al*. 2006).

Hypermutability of nucleoside diphosphate kinase (Ndk) mutants

Nucleoside diphosphate kinase is a ubiquitous enzyme present in many organisms ranging from bacteria to humans and catalyses the reversible transfer of the γ phosphates between nucleoside diphosphate and triphosphates. In *E. coli* there is a single copy of the gene, *ndk*, encoding this enzyme (Ndk) while in humans there are multiple copies, designated as *NM23-H1* to *NM23-H8* (Lacombe *et al*. 2000). Ndk is a key enzyme involved in the synthesis of dNTPs and NTPs needed for replication and transcription, respectively, as well as in maintaining a balance of nucleotide triphosphate pool sizes. In addition to their main role in the biosynthesis of nucleotide precursors, the *E. coli* and the human enzymes have been shown to have several other activities (for references, see Goswami *et al*. 2006). Aspects of DNA precursor metabolism and genomic stability have been reviewed recently (Mathews 2006).

Lu *et al*. (1995) reported that insertional disruption of *ndk* in *E. coli* was not lethal indicating that the gene is dispensible. The Ndk enzymatic activity of two independently isolated *ndk* insertional mutants was still ~15% of the wild-type cells suggesting that some other gene(s) could provide some degree of substitute Ndk activity. Loss of some potential substitute genes, namely, the ones coding for pyruvate kinases (*pfkA* and *pfkF*) and the gene for succinyl CoA synthetase (*scs*) did not abolish the viability of an *ndk* mutant showing yet other gene(s) could be involved (Lu *et al*. 1995). Subsequently Lu and Inouye (1996) showed that the viability of an *ndk* mutant is due to adenylate kinase. The *ndk* mutants displayed two important phenotypes: (i) they were moderately hypermutagenic; and (ii) there was an imbalance in the dNTP pool sizes, the levels of dGTP and dCTP being elevated 7 and 20 fold, respectively (Lu *et al*. 1995). Another interesting observation that followed was that the *ndk* mutants not only supported the development of phage T4, but that T4 infection corrected the imbalance in dNTP pool sizes as well as hypermutability (Zhang *et al*. 1996). Growth of *ndk* mutants under anaerobic conditions also mitigated their characteristic phenotypes (Zhang *et al*. 1996). These results led to the emergence of a strong belief that the mutator phenotype of *ndk* mutants could be due to the imbalance in dNTP pool sizes. Although single mutants in *ndk* are only moderate hypermutators, double mutants harbouring *ndk* and either *mutS* or *mutD* mutant alleles are very strong mutators (Miller *et al*. 2002; Hong *et al*. 2005). Transitions (A : T → G : C) and transversions (A : T → T : A) were elevated more than 1000-fold, as well as frameshifts in repeat runs. It was suggested that Ndk deficiency stimulated replication errors and editing defects which get exacerbated when the respective repair processes are also defective (Miller *et al*. 2002).

A recent report by Nordman and Wright (2008) has thrown new light on the mutagenic potential of Ndk deficiency. The above authors showed that the human homologue of *E. coli ndk*, namely, *NM23-H2*, complemented the mutator phenotype of an *E. coli ndk* mutant but neither corrected the dNTP pool imbalance nor restored Ndk enzymatic activity. Nordman *et al*. (2007) had earlier identified a transposon insertion in *ndk* that could alleviate the cold sensitive phenotype (inability to form colonies at 30°C) of a *dnaA*(*Cos*) mutant of *E. coli*, that is, *ndk-dnaA*(*Cos*) double mutants could form colonies at 30°C. Cold sensitivity could be restored by introducing plasmid-borne wild-type *ndk* gene. An *ndk-dnaA*(*Cos*) mutant which is able to grow at 30°C continued to be so with *NM23-H2*, indicating that the latter could not fully complement *ndk* deficiency (Nordman and Wright 2008). These observations negated the long held belief that dNTP pool imbalance and mutator phenotype of *ndk* mutants are functionally related. Nordman and Wright (2008) also made two more important observations: (i) double mutants harbouring *ndk* and *dut-1* (dUTP-ase defective) are extremely strong mutators (> 3000-fold stronger than *ndk* single mutants); and (ii) *ndk-dut-1-ung* (uracil DNA glycosylase defective) triple mutants are also strong mutators but not as strong as *ndk-dut-1* doubles (300-fold as against 3000-fold). These results have brought into focus the involvement of dUTP-ase and uracil DNA glycosylase in hypermutagenesis caused by Ndk deficiency.

Goswami *et al*. (2006) had earlier provided strong evidence for molecular and functional interaction between Ndk and Ung proteins (copurification, co-immuno-precipitation and stimulation of Ung activity by Ndk etc.). In particular, they showed that the uracil DNA glycosylase activity of purified Ndk protein, originally reported by Postel and Abramczyk (2003), and later questioned by Bennett *et al*. (2004) and Kumar *et al*. (2004), is not an intrinsic property of the Ndk protein but a consequence of Ndk–Ung interaction which, incidentally, lowers the *Km* of Ung for its substrate ~5-fold. On the basis of their own observations and those of Goswami *et al*. (2006), Nordman and Wright (2008) have proposed that Ndk functions at the interface of uracil excision repair and thymine biosynthesis, to prevent excessive accumulation of dUTP in the cell and consequent excessive incorporation of uracil into DNA. The exacerbation of the mutator activity of *ndk* mutants by the *dut* mutation and the

partial alleviation of this effect by the *ung* mutation support this view. They propose that a-basic sites which result from excision of misincorporated uracil from DNA could be responsible for the mutator phenotype of *ndk* mutants. For a detailed discussion see Nordman and Wright (2008).

Concluding remarks

The literature on mutators and hypermutability is formidably voluminous. Due to constraints of space, I had to restrict the discussions only to the mechanisms of the phenomenon. Other interesting and significant facets of hypermutability such as its evolutionary implications, issues relating to fitness, stability, competitiveness, implications in systematics and above all, the import of hypermutability on medical microbiological problems, have been left out from discussion. However, some important references have been cited (see Introduction). The human counterparts of some bacterial genes involved in hypermutability (mismatch repair genes, *ndk* etc.) have been implicated in diseases such as hereditary colorectal cancer, ovarian cancer, initiation and progression of tumours, metastasis etc. and these issues have not been covered in this review. However, some key references can be found in Miller (1996), Miller *et al*. (2002) and David *et al*. (2007). In efforts to present a comprehensive coverage, keeping the length as short as possible, some important reports, even on the topics discussed, might have been left out inadvertently as well as unavoidably. Again, due to constraints of space, only heritable mutators have been discussed. Tansient hypermutability, which underlies stress-induced mutagenesis (adaptive mutagenesis) has not been touched at all, not because it is less important but it is a topic which deserves separate reviews by itself. Moreover, stress-induced mutagenesis has been covered in many excellent reviews recently (Wright 2004; Roth *et al*. 2005; Foster 2007; Galhardo *et al*. 2007; Hastings 2007; Gonsalez *et al*. 2008).

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