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Hypermutation in bacteria and other cellular systems

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A temporary state of hypermutation can in principle arise through an increase in the rate of polymerase errors (which may or may not be triggered by template damage) and/or through abrogation of fidelity mechanisms such as proofreading and mismatch correction. In bacteria there are numerous examples of transient mutator states, often occurring as a consequence of stress. They may be targeted to certain regions of the DNA, for example by transcription or by recombination. The initial errors are made by various DNA polymerases which vary in their error-proneness: several are inducible and are under the control of the SOS system. There are several structurally related polymerases in mammals that have recently come to light and that have unusual properties, such as the ability to carry out 'accurate' translesion synthesis opposite sites of template damage or the possession of exceedingly high misincorporation rates. In bacteria the initial errors may be genuinely spontaneous polymerase errors or they may be triggered by damage to the template strand, for example as a result of attack by active oxidative species such as singlet oxygen. In mammalian cells, hypermutable states persisting for many generations have been shown to be induced by various agents, not all of them DNA damaging agents. A hypermutable state induced by ionizing radiation in male germ cells in the mouse results in a high rate of sequence errors in certain unstable minisatellite loci; the mechanism is unclear but believed to be associated with recombination events.

Keywords: adaptive mutation; DNA polymerases; bacteria, hypermutation in; transcription, effect on mutation; mismatch correction; translesion synthesis

1. INTRODUCTION

To consider the phenomenon of hypermutation we must first identify what is normal. It is customary (although not always appropriate) to measure spontaneous mutation rates under conditions of growth and reproduction, and to express them per gene, or base, or base pair replicated. Thus in bacteria, mutation rates are generally found to be between 10^{-10} and 10^{-9} per base pair replicated. There is nothing universal about these figures, however. Among organisms with DNA genomes, mutation rates per nucleotide incorporated vary by more than 10^4-fold (see table 1). Surprisingly, when expressed per genome, the rates for cellular and subcellular organisms all come out at around 0.003, and for multicellular organisms slightly more (Drake 1991; Drake *et al*. 1998). Genomes also vary by a factor of more than 10^4-fold and the larger the genome, the lower the mutation rate per base pair. Mutation rates per nucleotide are thus inversely related to genome size. This relationship, which shows that mutation rates are themselves highly evolved, is known as Drake's rule and from it evolutionary geneticists have made a number of important extrapolations. Organisms with RNA genomes do not comply with Drake's rule and are extremely highly mutable; the average mutation rate per genome for lytic RNA viruses is, for example, around 1.

In the early post-Watson–Crick days it was tempting to suppose that fidelity of DNA replication was largely dependent upon the hydrogen bonding of the two 'normal' base pairs, G with C and A with T. Time has shown that this pairing is no more than a rough preference, which is exploited by enzymes concerned with DNA processing. DNA polymerases themselves are responsible for the basic accuracy of replication. A replicative polymerase, such as the α-subunit of DNA polymerase III holoenzyme (Pol III) of *Escherichia coli*, can reduce the error rate for base substitutions to *ca.* 10^{-5} to 10^{-4} per base pair (Bloom *et al*. 1997), although this is dependent upon the base being replicated and the sequence context. Error rates for −1 frameshift mutations are higher (Mo & Schaaper 1996). Slightly lower rates (*ca.* 10^{-6}) have been reported for Pol III holoenzyme acting *in vitro* and *in vivo* (Fujii *et al*. 1999; Maor-Shoshani *et al*. 2000). Overall it may be inferred that base selection by the α-subunit contributes a factor of *ca.* 10^4–10^5 to the fidelity of genome replication (Bloom *et al*. 1997; Sloane *et al*. 1988). A glance at table 1, however, shows that a polymerase error rate of 10^{-5} or 10^{-4} is by no means enough to account for the fidelity of replication in *E. coli* (5.4×10^{-10} per base pair). To achieve rates such as this, two important devices have evolved to correct polymerase errors, or misincorporations.

The first of these is 'proofreading', which is carried out in extremely close proximity to the polymerase. The proofreading function is sometimes carried out by another domain of the polymerase polypeptide itself, as with bacterial DNA polymerase I (Pol I), and sometimes by a separate protein, as in bacterial Pol III where the polymerase function is carried out by the α-subunit and

Table 1. *Drake's rule concerning mutation rates per generation and genome size*

(Data from Drake *et al*. 1998.)

organism	genome size (bases or base pairs)	mutation rate per base	mutation rate per genome
phage M13	6.4×10^3	7.2×10^{-7}	0.0046
phage λ	4.9×10^4	7.7×10^{-8}	0.0038
phages T2 and T4	1.7×10^5	2.4×10^{-8}	0.0040
Escherichia coli	4.6×106	5.4×10^{-10}	0.0025
Saccharomyces cerevisiae	1.2×10^7	2.2×10^{-10}	0.0027
Neurospora crassa	4.2×10^7	7.2×10^{-11}	0.0030
Caenorhabdidis elegans	1.8×10^{7a}	2.3×10^{-10}	0.004[b]
Drosophila	1.6×10^{7a}	3.4×10^{-10}	0.005[b]
mouse	8×10^{7a}	1.8×10^{-10}	0.014[b]
human	8×10^{7a}	5×10^{-11}	0.004[b]

[a] For higher eukaryotes this is the effective genome size, i.e. that portion in which most mutations are deleterious.
[b] This is the mutation rate per effective genome.

the proofreading function by the ε-subunit. Essentially, proofreading is the action of a 3′- to 5′-exonuclease, which has a greater probability of excising a newly polymerized base that is mismatched than one that is correctly matched. *In vitro* work has shown that at least 92% of misinserted nucleotides are removed by the ε-subunit of Pol III, except where the next template base correctly matches the inserted mismatched base (Bloom *et al*. 1997). Overall, the presence of ε-subunit in bacterial Pol III typically reduces misincorporation frequencies to between 10^{-7} and 10^{-6} (Bloom *et al*. 1997; Fersht 1979; Fersht & Knill-Jones 1981, 1983*a*,*b*; Fersht *et al*. 1982). The efficiency of proofreading is determined in part by the polymerase that has undertaken the synthesis. Polymerase fidelity is a function not only of the probability of inserting a mismatched base, but of the ability of the polymerase to use the mismatched base as a primer for further synthesis. A polymerase that is reluctant to continue synthesis on a mismatched primer terminus will allow much more time for proofreading to act than one that continues synthesis and so hides the mismatched base from the proofreading exonuclease. This property of a polymerase may be quite independent of its intrinsic misincorporation rate.

Second, quantitatively more important than proofreading is 'mismatch correction', which occurs some way behind the replication fork. In this, one or more nucleases removes a section of the newly synthesized strand in a region that contains a mismatch. In bacteria the most important mechanism is one that removes a long patch (around 10^3 nucleotides) of newly synthesized DNA and allows a second attempt at polymerization. Mismatch correction operates not only on mismatched bases but also on small frameshifts, which cause one strand to loop out. The proteins involved in this pathway are conserved from bacteria to humans and the mechanism is an important defence against cancer (Kolodner 1996). To be effective, mismatch correction must not only recognize the presence of a mismatch but also distinguish which strand is parental and which is newly synthesized. In *E. coli* this is achieved by means of a methylation tag. Soon, but not immediately, after polymerization a methyl group is attached to adenine residues at specific sequences in the DNA. Until this is done, the newly synthesized strand can be recognized by the absence of the methyl groups and there is thus a window of time in which mismatch correction can take place (Marinus & Morris 1974). Other bacteria and higher organisms have other ways of distinguishing newly synthesized strands of DNA from parental, most of which remain cloaked in mystery (Jiricny 1998).

The combined operation of mismatch correction following replication and proofreading enables the overall error frequency in *E. coli* to be reduced to below 10^{-9} per base pair replicated.

2. HYPERMUTATION

A state of hypermutation can thus be achieved in one or a combination of three ways: (i) by increasing the misincorporation rate of the DNA polymerization step, (ii) by abrogating proofreading, or (iii) by abrogating mismatch correction. This can occur, for example, through heritable defects in the genes directly responsible for these three processes or in genes that cause errors in translation that indirectly lead to the synthesis of aberrant proteins involved in these processes (cf. Slupska *et al*. 1996). Such strains are inherently hypermutable and are known as 'mutator strains'; the alleles responsible are known as 'mutator alleles' (Horst *et al*. 1999). Although mutators have given us the genetic tools to tackle the mechanisms of mutation and replication fidelity, they will not be considered further here since we are primarily concerned with processes that cause a temporary state of hypermutation in a high proportion of a population. Transient mutator bacteria may also arise at a very low frequency in a population due to abnormal polymerases and fidelity proteins that have arisen as a result of mistranscription or mistranslation. Ninio (1991) has calculated that such transient mutators might produce 10% of the single mutations and more than 95% of double mutations that arise simultaneously in a population.

In the hypermutation that occurs during B-cell maturation the error frequencies are high. An average value of $4–10 \times 10^{-4}$ per base pair replicated has been calculated (Allen *et al*. 1987; Berek & Milstein 1987; Weigert 1986), but this may underestimate the actual frequency in certain situations where values of *ca.* 10^{-2} may occur (Yelamos *et al*. 1995). The implication of this is

Table 2. *DNA polymerases of* E. coli

polymerase	gene	functions
Pol I	*polA*	DNA repair and ancillary role in replication
Pol II	*polB*[a]	reassembly of stalled replication complexes and bypass of certain lesions
Pol III	*dnaE* (*polC*)	chromosomal replication and certain repair events
Pol IV	*dinB*[a]	untargeted mutagenesis and bypass of certain lesions
Pol V	*umuC*[a]	targeted mutagenesis, translesion synthesis

[a]These are expressed as part of the SOS system, dependent on genes *recA* and *lexA*.

that if the hypermutation is due simply to errors occurring during polymerization, not only must both proofreading and mismatch correction be inoperative, but the polymerase step must be far more error-prone than that mediated by any normal replicative polymerases. As will become apparent, none of the known bacterial DNA polymerases has error rates approaching 10^{-2}, even those responsible for elevated mutation rates in bacteria and bacteriophages. Some mammalian polymerases, however, may do so (Dominguez *et al*. 2000; Matsuda *et al*. 2000). In this review the focus will be primarily (though not exclusively) on bacteria because much has been happening in the last year or two and there has been something approaching a paradigm shift in our understanding of mutation processes. During this time the number of known bacterial DNA polymerases has increased from three to five, and the functions of the two new enzymes plus one of the established enzymes, previously without a known effect on cell phenotype, have become increasingly clear. Table 2 lists these polymerases and some of their properties. We shall next consider some situations where there is evidence for hypermutation.

3. GENOME REARRANGEMENTS

Given that mammalian immunoglobulin genes have evolved sophisticated machinery for rapidly generating variable sequences for responding to antigenic stimulation, it is not surprising that pathogenic microbes have themselves developed analogous mechanisms for changing their antigenic profile and thus evading the host's defences (Moxon *et al*. 1994; Seifert & So 1988). A variety of mechanisms exist showing that the ingenuity of the microbe can match that of the host. It has been proposed that bacterial genes can be divided into 'contingency' genes that are potentially highly mutable and 'housekeeping' genes that are not (Moxon *et al*. 1994). Wherever they have been examined the 'mutations' in contingency genes have proved to be rather specific and have not involved error-prone DNA polymerization. They are generated by various mechanisms including gene conversion, site-specific recombination, oligonucleotide repeats, homopolymeric tracts, site-specific transposition and site-specific methylation (Moxon *et al*. 1994; Seifert & So 1988). Such processes are part of a much broader picture that has been termed 'phenotypic metastability' and includes alternative expression (of the simple switching type) of individual genes or small groups of genes, variation in protein composition controlled at the level of transcription, and phage transposition (Golovlev 1998). The essence of all these cases is that there are particular genetic changes that are advantageous in certain alternative conditions frequently experienced by the species. Mechanisms have therefore evolved to generate specifically these changes at relatively high rate. They are clearly distinct from an elevated rate of conventional mutations like base pair substitutions and small frameshifts within specific genes such as occurs during B-cell maturation.

4. HYPERMUTABLE STATES: THE SOS SYSTEM

The most immediate stress that is experienced by the genome is structural damage to itself. DNA is subject to reaction with many other chemicals, particularly water, oxidizing agents and electrophiles, and with radiation. Ultraviolet radiation from the sun is nature's most ubiquitous exogenous hazard to nucleic acids; ultraviolet radiation of slightly shorter wavelength from mercury vapour lamps has been our most useful laboratory tool in unravelling the consequences of damage to DNA nucleotides. The fact that such damage is usually confined to one of the two strands at any site and that there remains an undamaged strand with complementary information has allowed cells to develop a variety of ways to remove or tolerate damage before they encounter a replication complex. They may cut the damage out and fill the gap using the undamaged strand as a template, or they may use recombination to rearrange the DNA in such a way that it may continue to function until such time as the damage can be cut out. Occasionally, however, there will be damage that cannot be (or is not) dealt with in either of these ways, perhaps because the opposite strand has itself sustained some other damage nearby. The cell then takes a compromise position and allows replication to occur at the risk of errors or mutations being introduced into the newly synthesized strand. Because this is something that would be dangerous in normal circumstances, the system that permits synthesis past damage (translesion synthesis) does not normally function in the absence of DNA damage in *E. coli*. It is in fact part of the SOS response (controlled by the *recA* and *lexA* genes) that is induced only when a significant amount of DNA damage is detected. The situation may be different in human cells.

A comprehensive discussion of DNA repair and the SOS mutagenesis system up to about 1995 can be found in Friedberg *et al*. (1995). At that time the understanding was that to insert a base opposite a damaged template site the cell used the normal replicative DNA polymerase Pol III α-subunit and a complex of two SOS-inducible proteins, UmuC and UmuD′, the latter being a post-transcriptionally modified form of the product of the *umuD* gene. UmuC and UmuD′ were thought in some way to lower the stringency of Pol III to permit it to carry out translesion synthesis. Very recently, however, it has become clear that the UmuD′,C complex is itself a DNA polymerase—now designated DNA polymerase V (Pol V)—and that it is able

to catalyse translesion synthesis, the insertion of bases opposite damaged template sites (Reuven *et al*. 1999; Tang *et al*. 1998, 1999). Our understanding of this process is now rapidly progressing; both Pol III and Pol V are needed for optimal rates of translesion synthesis, although it is still not clear exactly what role Pol III plays.

In addition to catalysing translesion synthesis, Pol V is a low fidelity polymerase when acting on an undamaged template. *In vitro* studies indicate that polymerization by Pol V is highly dependent upon the presence of RecA protein and has an error rate of 10^{-4} to 10^{-3} (Maor-Shoshani *et al*. 2000; Tang *et al*. 1999). Pol V has low processivity, adding six to eight nucleotides before dissociating (Tang *et al*. 2000). While the inducibility and low processivity of Pol V restrict its opportunity to defile the genetic message under normal circumstances, there is circumstantial evidence that when Pol III is defective and Pol V is constitutively induced, the latter can accomplish significant chromosomal replication resulting in hypermutation (Ruiz-Rubio & Bridges 1987). It has long been considered that the UmuD′ and UmuC proteins were necessary for extension from a primer terminus opposite a damaged base and that this might be independent of the insertion of a wrong base opposite the lesion (cf. Bridges & Woodgate 1985).

Now we know that Pol V is not the only error-prone polymerase under SOS control. It has been known for more than 50 years that if non-irradiated λ-phage were allowed to infect bacteria that had been exposed to a heavy dose of ultraviolet light, there was a massive induction of mutations in the phage (Jacob 1954). This hypermutator effect on undamaged DNA could also be seen if the SOS system was induced by means of a temperature-sensitive mutation in the *recA* gene and the mutations arose sporadically over many cycles of phage replication (Ichikawa-Ryo & Kondo 1975; Von Wright & Bridges 1980). The mutations were found to be predominantly frameshifts (Wood & Hutchinson 1984) and were dependent on *dinB*, a gene of unknown function under the control of the SOS system (Brotcorne-Lannoye & Maenhaut-Michel 1986).

The product of the bacterial *dinB* gene, a homologue of *umuC,* has now been shown to be a DNA polymerase and has been designated DNA polymerase IV (Pol IV) (Wagner *et al*. 1999). Like Pol V, it is distributive rather than processive and devoid of proofreading activity. While more error-prone than the replicative polymerases such as Pol III it is still five to ten times more accurate than Pol V (Tang *et al*. 2000) and tends to generate −1 frameshifts rather than base pair substitutions (Wagner *et al*. 1999). The role of Pol IV in the cellular economy of *E. coli* is still something of a mystery; there has so far been no convincing evidence for any such action on the undamaged bacterial chromosome itself but this may be because this possibility has not yet been examined in an appropriate experimental system. Its primary role may even be to assist in dealing with damaged DNA since evidence is emerging that it may assist in translesion synthesis at specific lesions, for example at benzo[a]pyrene adducts (R. Fuchs, personal communication) and at sites where two guanines are linked by cisplatin (H. Ohmori, personal communication).

It will have become apparent that in addition to being able to deal with non-repaired DNA damage, a cell in which the SOS system is induced may also generate a great deal of genetic variability due to the infidelity of various polymerases acting on undamaged DNA. Indeed, there is a view that the primary (evolutionary) function of this hypermutable state is to generate variability under stressful conditions rather than to enhance survival. This view would carry more weight if there were stressful conditions in which the SOS system was induced where there was no associated damage to DNA. The induction that occurs in ageing colonies on agar plates might be one such condition (Taddei *et al*. 1997), but oxidative damage to DNA is also likely to be associated with such a state.

A human homologue of the bacterial *dinB* gene has been identified and its product purified. It is also a polymerase and has been designated Pol θ (Johnson *et al*. 2000). Like bacterial Pol IV it is unable to carry out translesion synthesis opposite a *cis-syn* thymine–thymine dimer, a (6-4) photoproduct, or an abasic site, and its error rate on undamaged DNA is *ca.* 10^{-4} to 10^{-3}. Several DNA polymerases structurally related to Pol V have been found in eukaryotes. Human DNA polymerase eta (Pol η) is defective in variant forms of the hereditary disease xeroderma pigmentosum, a sunlight-sensitive cancer-prone condition. Because it inserts primarily adenines opposite thymine dimers formed by ultraviolet radiation it was considered to be able to carry out error-free translesion synthesis. With other lesions it has proved to be less successful in inserting the correct nucleotide but when the inserted nucleotide is incorrect it is unable to use it as a primer for further elongation (Masutani *et al*. 2000), a property that contributes to its effective fidelity.

Pol η has no proofreading activity and has proved to possess a lower fidelity on undamaged templates than any other template-dependent DNA polymerase studied. Depending on the precise nature of the template, it makes one base substitution error for every 18–380 nucleotides polymerized (Matsuda *et al*. 2000). Even less accurate than Pol η is human DNA polymerase mu (Pol μ), which is not template dependent and has intrinsic terminal deoxynucleotidyl transferase (TdT) activity. In the presence of a template strand there was preferred insertion of complementary nucleotides, although with low discrimination values. Pol μ mRNA was found to be preferentially expressed in human peripheral lymphoid tissues and examination of databases indicated that a large proportion of expressed sequence tags corresponding to Pol μ derived from germinal centre B cells. Pol μ is therefore a good candidate to be the polymerase responsible for hypermutation in immunoglobulin genes (Dominguez *et al*. 2000).

5. NUTRITIONAL STRESS

It is arguable that the commonest stress experienced by free-living cells is nutritional—indeed most such cells probably spend most of their time waiting for their next meal to come along. During times of starvation, DNA replication is minimal and yet many years ago it was shown that mutations can still arise (Ryan *et al*. 1961). DNA synthesis is hard to measure during starvation since the cells may recycle nucleotides from degraded nucleic acids rather than use exogenous labelled nucleotides. When Foster (1993) reviewed studies using the conventional

methodology with exogenous label she concluded that DNA synthesis was between 0.5 and 5% per genome per day. When set against this small amount of synthesis, calculated mutation rates could be very substantially above those observed during logarithmic growth. Using an indirect method involving an endogenously generated label, the rate of DNA synthesis appeared to be higher—of the order of 2% per genome per day (Bridges 1996). Whether this represents genome turnover in non-dividing bacteria or replication and division of a minority of the population is not clear. A general impression from the available literature consistent with our own experience is that hypermutation in starved cells is greatest when there is some leakiness, i.e. some residual growth (Jayaraman 1995; Mittler & Lenski 1992; Timms & Bridges 1998), although there does not seem to be a direct quantitative relationship (Galitski & Roth 1996; Prival & Cebula 1996). Systems in which there is no leakiness at all rarely show high rates of mutation. This is perhaps not surprising since there must be some energy requirement even for mutation pathways that are seen only under starvation conditions. Nevertheless, when expressed in terms of the amount of DNA replicated, mutation rates under some conditions of nutritional stress can be very high—a genuine hypermutation situation.

The most telling argument that mutational pathways under conditions of nutritional stress are different from those seen during logarithmic growth is that the spectrum of mutations is almost always completely different. The experiments under consideration are those carried out under conditions of non-lethal selection, i.e. the bacteria (or most of them) are alive but they must mutate in order to grow and form a visible colony. This process is often loosely termed 'adaptive' mutation and it is frequently seen when bacteria auxotrophic for an amino acid are incubated in a medium lacking that amino acid but otherwise able to support growth, or when bacteria are incubated with a carbon energy source that they cannot metabolize. In many of the early experiments of this type the fact that mutations were not found at loci that did not confer the ability to grow led to the idea that bacteria possessed the ability to mutate only at those loci that carried a potential selective advantage (Cairns *et al*. 1988; Hall 1990). This idea of 'directed' mutation naturally did not find favour with evolutionary biologists, even though there were at least some perfectly acceptable mechanisms by which it could happen (cf. Bridges 1994). Latterly, however, it has become clear that if the search is thorough enough mutations can be seen at loci without conferring a selective advantage (Foster 1997; Torkelson *et al*. 1997). Thus the hypothesis of 'directed' mutation remains unsubstantiated and other explanations must be found for the different mutation spectrum observed in growing and non-growing cells.

6. DNA DAMAGE

(a) *Miscoding lesions*

We have so far considered only polymerase infidelity as the primary source of spontaneous mutations, albeit filtered through various mechanisms for increasing fidelity. The other possible primary source is damage to DNA through endogenously generated mutagens (cf. Bridges 1996, 1998). It would not be surprising if under conditions of nutritional stress, when DNA replication is minimal, DNA damage assumed a more important role in the generation of mutations. So it has turned out. Active oxidative species, such as peroxides, superoxide and singlet oxygen, are ubiquitous in aerobic organisms and they have the potential to interact with the bases in DNA to form lesions. While many types of DNA lesion result in loss of coding ability, those formed by oxidative species frequently result in a change in coding ability. Guanine normally pairs with cytosine, but 8-oxo-7,8-dihydrodeoxyguanine (8-oxoguanine) also pairs readily with adenine. This, if not corrected, will lead to a transversion from a G:C base pair to a T:A base pair. That this indeed happens under starvation conditions in several systems has now become clear. The rate of mutation is slightly elevated in *mutM* bacteria, appreciably elevated in *mutY* bacteria, and greatly elevated in *mutM mutY* double mutants (Bridges 1995*a*; Bridges *et al*. 1996). MutY and MutM proteins determine glycosylases that respectively remove adenine mispaired with 8-oxoguanine and 8-oxoguanine paired with cytosine. Overproduction of these proteins when their genes are on a multicopy plasmid reduces the rate of mutation by about half, indicating that the 8-oxoguanine is involved in mutation in non-growing cells when these enzymes are present at normal levels.

Oxidized bases are not the only miscoding lesions to be formed in DNA by endogenous processes. Rebeck & Samson (1991) concluded that there was an endogenous alkylating agent in non-dividing cells capable of reacting with DNA and giving rise to O-6-alkylguanine and O-4-alkylthymine, both of which are capable of miscoding during subsequent DNA replication. Similarly nitroso compounds have been inferred to alkylate DNA in non-dividing cells (Taverna & Sedgwick 1996). Both the above reports were based on observations with strains that were deficient in repair of alkylated bases and it is not clear whether mutations arise in this way in strains that are competent for repair.

In considering hypermutation, therefore, we must think not only about how errors may arise during DNA polymerase activity and how fidelity mechanisms may be abrogated, but also about how the accumulation of lesions in DNA may lead to miscoding at a subsequent replication. With miscoding lesions the nature of the polymerase that is involved in the miscoding may be relatively unimportant since the miscoding is a characteristic of the lesion rather than the polymerase. There is little to suggest that unusual polymerases are involved—in two small studies, deletion of Pol I or a reverse transcriptase carried by a defective prophage had no effect (Bridges 1993, 1995*b*).

If one may generalize, it seems that oxidative damage tends to become important when DNA replication is minimal and cells are not actively growing. It does not seem likely to contribute to somatic hypermutation in immunoglobulin genes, which occurs in cells that are actively growing. Moreover, the spectrum of mutations generated by oxidative damage (transversions, frameshifts, small deletions) is clearly different from the transition-dominated spectrum in immunoglobulin gene hypermutation.

(b) *Non-coding lesions*

Miscoding lesions have dominated thinking about DNA damage in hypermutation in bacteria under nutritional stress largely because non-coding and distorting lesions, which are perhaps more typical of DNA damage, require the translesion synthesis potential of the SOS system to give rise to mutations. As discussed in § 4, the SOS system is inducible and requires protein synthesis, so its contribution to hypermutation in non-dividing cells was expected to be minimal. This may, however, prove to be a mistaken assumption. It has recently been reported that the SOS system can become induced in ageing colonies on agar plates and that this may be associated with an elevated mutation frequency (Taddei *et al*. 1997). The *umuD,C* genes (specifying Pol V) were not required for this mutagenesis—Pol IV appears to be a more likely candidate. Pol V has, however, been found to be required for the production of G:C to C:G transversions in a strain of *E. coli* lacking the glycosylase that removes adenine that is mispaired with 8-oxoguanine. These transversions are found only in starved bacteria and not in growing bacteria (Timms *et al*. 1999; Zhang *et al*. 1998). While the existence of this pathway has only been demonstrated in a *mutY* repair-deficient strain, it suggests that Pol V-dependent hypermutation pathways may be more prevalent under starvation conditions than has hitherto been recognized.

7. POTENTIALLY LETHAL SELECTION

The demonstration of adaptive hypermutation under conditions of nutritional stress is made possible because the population is viable, or at least contains a subpopulation that is viable. Does the process exist in populations that are being actively killed, for example by an antibiotic? Such a possibility would have very significant implications for the emergence of antibiotic-resistant strains in clinical or veterinary practice. There are obviously practical difficulties in performing experiments to investigate this possibility since the number of viable bacteria is declining all the time and in consequence the statistical power to detect antibiotic-resistant mutants is progressively lost. There appears to be only one report of adaptive mutation to antibiotic resistance and it concerns the action of concentrations of ciprofloxacin marginally above the minimal inhibitory dose on *E. coli* (Riesenfeld *et al*. 1997). While the number of mutations per viable cell increases dramatically during treatment with the antibiotic, the result is based on a very small number of actual resistant colonies and must be regarded with some degree of caution. Notwithstanding, given the potential clinical importance of the phenomenon, further studies in this area are urgently needed.

8. HYPERMUTATION IN AN EPISOMAL GENE IN *E. COLI*

The most high profile work on adaptive mutation under nutritional stress has been carried out with the FC40 strain of *E. coli* and is worth considering since it appears to show a high degree of specificity in so far as it is manifest only with a particular mutant stressed in a particular way. FC40 contains a chromosomal *lac* deletion and an F′Lac episome with the *lacI33* allele that reverts to Lac^+, typically by −1 frameshifts in a short run of Gs. When incubated on minimal lactose plates, Lac^+ revertants arise over several days (Cairns & Foster 1991). This particular system differs from all others so far reported in being dependent upon the *recA,B,C* genes (Cairns & Foster 1991; Harris *et al*. 1994) and on the sexual potency of the bacteria (Foster & Trimarchi 1995; Galitski & Roth 1995; Radicella *et al*. 1995), i.e. it requires the ability to undergo conjugational DNA synthesis (although conjugation itself is not necessary). It is generally thought that this is a recombination-driven process in which the recombination triggers DNA synthesis. When the *lacI33* gene is on the chromosome and not on the episome the mutation rate is much lower and is not *recA* dependent (for a review, see Foster 1999). Nevertheless, there are certain sites on the bacterial chromosome that show hypermutation along with the episomal *lac* gene, presumably because they are recombination hot spots (Bull *et al*. 2000).

Initially the system attracted much attention since early results suggested that the process really was directed, i.e. mutations only occurred in *lacI*, the gene under selection. However, subsequent results have shown that other loci (but not all loci) also mutate at a high rate when FC40 is placed under lactose selection (Foster 1997; Torkelson *et al*. 1997). It appears that the mutations arise in a small subpopulation of cells in a hypermutable state, a concept first proposed as a mechanism for adaptive mutation by Hall (1990). One estimate is consistent with a rate of 5×10^{-3} per cell per day in a subpopulation of 10^{-5} to 10^{-4} of the whole population (Torkelson *et al*. 1997). Although this rate is clearly very high, it is not possible to ascribe a quantitative rate per base replicated since the amount of plasmid replication under selection conditions is not known for the whole population, let alone the hypermutable subpopulation.

Mismatch correction is known to decline as cells move from growth phase to the non-dividing state (Bregeon *et al*. 1999; Feng *et al*. 1996; Harris *et al*. 1997*b*). Since the mutations seen in FC40 undergoing adaptive mutation are of the type that would be susceptible to mismatch correction (Longerich *et al*. 1995), it could explain at least part of the hypermutability if mismatch correction was abrogated (Foster & Trimarchi 1994; Rosenberg *et al*. 1994). While the depletion of mismatch correction under these conditions has been controversial, there now seems to be general agreement that mismatch correction is at a low level, at least in the hypermutable subpopulation.

While the absence of mismatch correction is important, is an error-prone polymerase involved in making the adaptive mutations? Studies with an allele of *dnaE*, which gives rise to a DNA polymerase III (Pol III) with greater than normal fidelity, showed that it also conferred a decrease in the rate of adaptive mutation, suggesting that Pol III was responsible for many of the initial mutation events (Foster *et al*. 1995; Harris *et al*. 1997*a*). The fact that adaptive mutation in FC40 is *recA* dependent might, of course, indicate the involvement of polymerases under SOS control. Recent work has indicated that adaptive mutation in FC40 is indeed under SOS control (McKenzie *et al*. 2000). Of the three polymerases under SOS control, Pol V (UmuD′,C) can be excluded since

bacteria unable to process UmuD to its active form shows normal adaptive mutation (Cairns & Foster 1991) and a deletion of *umuD',C* also has little effect (McKenzie *et al*. 2000). Pol II can also be excluded since bacteria carrying a deletion of *polB* show an enhanced rate of adaptive mutation (Escarceller *et al*. 1994). Indeed, this result suggests that when it is present Pol II competes with the mutation-generating polymerase(s) and is more accurate than them. That leaves Pol IV, the product of the *dinB* gene, and recent work indicates an involvement of this polymerase (H. Ohmori and P. L. Foster, personal communication). What is not yet clear is whether the frameshift mutations are simple polymerase errors or whether they are triggered by the presence of lesions in the template strand. Oxidative lesions produced by singlet oxygen in phage DNA are known to be able to trigger single base deletions when the DNA is transfected into cells (Decuyper-Debergh *et al*. 1987; Van den Akker *et al*. 1994) and expression of endogenous carotenoids, which scavenge singlet oxygen, is associated with a significant reduction in the rate of adaptive mutation in this system (B. A. Bridges, P. L. Foster and A. R. Timms, unpublished data).

Adaptive mutation in the FC40 system is of particular interest in the context of somatic hypermutation in immunoglobulin genes as it shares the property of being specific to certain DNA sequences and manifesting an extremely high mutation rate.

9. HYPERMUTATION AS A FUNCTION OF TRANSCRIPTION

The adaptive hypermutation discussed above occurs over days and under conditions of severe nutritional stress. The situation that occurs towards the end of a phase of growth is rather different in that there is still plenty of metabolic capability within the cell, yet it is already beginning to respond to changing nutritional conditions by initiating changes in gene expression. Given that a variety of lines of evidence implicate transcription in the site-directed somatic mutation in immunoglobulin genes (Betz *et al*. 1994; Maizels 1995; Storb *et al*. 1998), it is of interest that increased transcription in micro-organisms is frequently associated with a burst of hypermutation. This is particularly apparent in yeast. For example, Korogodin *et al*. (1991) showed that a decrease in the amount of adenine or leucine in the medium with the associated transition from repression to derepression of the genes responsible for biosynthesis of these metabolites resulted in a 15- to 150-fold increase in the reversion rates of the *ade2* and *leu2* genes, respectively (Korogodin *et al*. 1991). The reversion rate at suppressor loci varied little. When a *lys2* frameshift allele was placed under the control of an inducible promoter, a 35-fold increase in mutation rate occurred when the gene was transcribed at a high level, whereas reversion rates in a non-inducible *lys2* allele were not affected (Datta & Jinks-Robertson 1995). Further genetic analysis of this phenomenon has recently been published (Morey *et al*. 2000).

If cells have evolved a mechanism for derepressing preferentially those genes that are needed to compensate for deficiencies in the nutrient environment then the result will be a genuine directed mutation phenomenon. Mutations will be specifically targeted in those genes that confer a selective advantage, although the mutational events within those genes will be stochastic and random. This line of argument has been pursued by Wright and her colleagues (Wright 1997; Wright *et al*. 1999). In *E. coli*, starvation for leucine triggers metabolic activities that specifically target the *leu* operon for derepression. Derepression of the *leu* operon is a prerequisite for its activation by guanosine tetraphosphate, a signal molecule that accumulates in response to nutritional stress (the stringent response). A qualitative correlation was established between *leuB* mRNA abundance and the rate of reversion of a *leuB* allele (figure 1). Moreover, when the *leu* operon was placed under the control of a *tac* promoter and induced with isopropyl-D-thiogalactoside, there was an increase in both *leuB* mRNA abundance and *leuB* reversion rates (Wright *et al*. 1999).

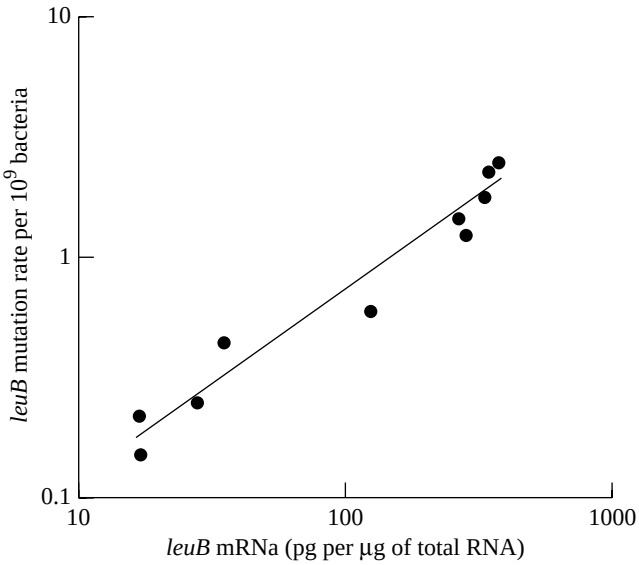

Figure 1. Correlation between *leuB* mRNA levels and reversion rates at the *leuB* locus (data from Wright *et al*. 1999).

Of course, the mutation rates shown in figure 1 are still low in comparison with immunoglobulin hypermutation rates, but they are occurring in a situation where proofreading and mismatch correction are still operating. Nevertheless, this study shows that specific derepression can be used as a device for targeting a specific sequence for hypermutation. How widely this device is used remains to be seen.

10. OTHER EXAMPLES OF SPECIFIC TARGETING

There are a few interesting examples of mutator activity at specific loci that are worthy of mention, even though they are strictly outside the remit of this review, which focuses on temporary non-genetic hypermutability. There are mutant forms of three proteins that confer a preferential or specific mutator effect in the region of their genes. The *recA1202* allele codes for a RecA protein that is a general mutator, elevating the spontaneous mutation frequency *ca*. 100-fold (Tessman & Peterson 1985). In the region of the *recA* locus, however, the frequency is increased 10^4- to 10^5-fold (Liu *et al*. 1993; Liu

& Tessman 1990). Subsequently, Liu *et al.* (1997) identified two further genes with alleles that conferred a preferential mutator activity in their own sequence and in adjacent sequences. One was an allele of the mismatch repair gene *mutS*, the other appeared to be a putative malate oxidoreductase gene. The mechanism behind the specificity of the mutator activity of these alleles is not known but the authors speculate that the local concentration of gene products during transcription of the gene is high and that the gene products in some way interfere with fidelity mechanisms operating on the genes nearby.

11. ANOTHER HYPERMUTABLE STATE IN MAMMALS

While the SOS system represents the prototype hypermutable state in bacteria, it does not normally persist indefinitely—once DNA damage is reduced to a low level, repression of SOS ensues. Persisting hypermutability in eukaryotes, however, has a long history. In earlier work the phenomenon was called replicating instability (for discussion, see Auerbach 1976). Subsequently, Stamato *et al.* (1987) observed the induction of glucose-6-phosphate dehydrogenase-deficient mutants in cultured Chinese hamster cells over at least eight to ten generations and of 6-thioguanine-resistant mutants over at least ten to 14 cell generations after treatment with ethyl methane sulphonate. These results suggested that the treatment had induced a heritable mutator phenotype in a substantial proportion of the population, a hypothesis that was supported by observations that a similar hypermutable state could be induced in Chinese hamster cells by X-rays and that it could persist for up to 95–100 population doublings (Chang & Little 1992).

Another example is afforded by cross-links formed by exposure of mouse lymphoma cells to ultraviolet A in the presence of 8-methoxypsoralen. There was an enhancement of mutation frequency per cell per generation that persisted for up to 11 generations (Boesen *et al.* 1992). Because cross-links are non-persistent lesions that will generate mutations only during the first replication after treatment, and because there were far too many mutations for the number of cross-links, most of the mutations must have arisen as a result of some persisting hypermutable state triggered (presumably) by the cross-links. A smaller effect was also seen with ultraviolet A alone or 8-methoxypsoralen alone. Unlike X-rays, methyl methanesulphonate, and ultraviolet A and 8-methoxypsoralen together, 8-methoxypsoralen alone does not damage DNA, so the induction of a hypermutable state is not confined to DNA-damaging agents. Herrlich (1988) suggested that hypermutability might be induced by signal transduction as part of a stress response. This suggestion is supported by the observation that the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate, known to affect signal transduction pathways, also induced a persisting hypermutable state without any direct induction of mutations (Boesen *et al.* 1991). Results such as the above indicate that hypermutable states can be triggered in cells outside the immune system. The mutation rates, however, are well below those encountered in the variable regions of immunoglobulin genes.

Perhaps the most interesting recent development in this area has concerned the induction of minisatellite mutation in the male germ cells of mice. Unstable minisatellites are repetitive sequences in DNA that are spontaneously unstable during germ cell development although they are stable during somatic cell division. Recombination processes associated with meiosis have been implicated in their formation. Sadamoto *et al.* (1994) reported the induction of new alleles of the unstable satellite *Ms6hm* in the offspring of C3H/HeN males exposed to gamma radiation. The authors argued that the frequency of *Ms6hm* mutations was too high to be accounted for by the direct production of radiation damage in the locus. Subsequently the same group reported a dose–response for irradiation of spermatids that showed a plateau above 2 Gy (Fan *et al.* 1995) and obtained a similar effect with Cf-252 radiation (35% gamma rays and 65% neutrons) for both spermatids and spermatogonia (Niwa *et al.* 1996).

A parallel set of observations has come from Dubrova and his colleagues using CBA/H mice (Harwell colony). Their results confirm the conclusion of the Japanese group that unstable minisatellite mutation is an untargeted process, i.e. that it occurs at sites that are not the sites of initial radiation damage (Dubrova *et al.* 1993, 1998*a*,*b*). They conclude that there are two associated processes leading to radiation-induced minisatellite mutation—structural damage induced elsewhere in the genome or in other sensor molecules and, subsequently, indirect mutation at satellite loci.

The Dubrova group have recently extended their observations to fission neutrons (Dubrova *et al.* 2000). Furthermore, they made the important observation that an elevated mutation rate also occurs in the non-irradiated germ cells of males born to irradiated fathers, in part attributable to germline mosaicism, and there was some evidence that an elevated rate also applied to DNA from the grandmaternal line that had never been irradiated. The authors argue that their results imply the existence of an exposure signal transmissible through sperm that subsequently destabilizes repeat DNA. What is not known is whether non-repetitive (i.e. coding) DNA is also susceptible to the hypermutable state and whether this state can be triggered by agents or stresses other than X-rays. The minisatellite mutations occurring in male germ cells are insertions and deletions and thus different in kind from the base changes seen in antibody genes. Nevertheless, the phenomenon is significant in so far as it demonstrates that hypermutation processes in healthy animals are not necessarily confined to immunoglobulin genes.

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