Minireview

Stationary phase mutagenesis: mechanisms that accelerate adaptation of microbial populations under environmental stress

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Summary
Microorganisms are exposed to constantly changing environmental conditions. In a growth-restricting environment (e.g. during starvation), mutants arise that are able to take over the population by a process known as stationary phase mutation. Genetic adaptation of a microbial population under environmental stress involves mechanisms that lead to an elevated mutation rate. Under stressful conditions, DNA synthesis may become more erroneous because of the induction of error-prone DNA polymerases, resulting in a situation in which DNA repair systems are unable to cope with increasing amounts of DNA lesions. Transposition may also increase genetic variation. One may ask whether the rate of mutation under stressful conditions is elevated as a result of malfunctioning of systems responsible for accuracy or are there specific mechanisms that regulate the rate of mutations under stress. Evidence for the presence of mutagenic pathways that have probably been evolved to control the mutation rate in a cell will be discussed.

Introduction
In nature, microbial populations are constantly confronted by variable and stressful environments. In most environments, such as in water and soil habitats, the stress conditions include, for instance, a generalized scarcity of nutrients (C, N and P sources), oxygen or water, non-optimal pH and temperature (van Veen et al., 1997 and references therein). It is widely assumed that carbon availability is the most common limiting factor for microbial growth in soil (Aldén et al., 2001; Koch et al., 2001). Studies of microbial activity in vivo indicate that growth of microbes in nature is restricted, although periods of inactivity alternate with periods of sporadic growth resulting from small amounts of heterogeneously distributed nutrients and energy sources (van Overbeek et al., 1995; van Veen et al., 1997; Söderberg and Bååth, 1998; Sternberg et al., 1999; Mascher et al., 2000; Ramos et al., 2000; Aldén et al., 2001; Koch et al., 2001). Nutrients may become available locally from decaying plant and animal material or as plant root exudates. So, spatial and temporal heterogeneity of microbial activity has been observed in the rhizosphere (Heijnen et al., 1995; Rattray et al., 1995; Maloney et al., 1997; Ramos et al., 2000). However, the growth rate of rhizosphere-associated bacteria decreases rapidly once the population reaches a steady-state level (Ramos et al., 2000). Results from Gsell et al. (1997) have indicated that the physiological activity of bacteria in nature varies seasonally, although the overall bacterial community structure and biomass do not change significantly. The mean values for bacterial turnover times in different soils using the thymidine and leucine incorporation data were found to be around 6 days but varied largely as a function of soil type (Bååth, 1998). For aquatic habitats, Kramer and Singleton (1992) have observed that the physiological state of Vibrio cells is characteristic of starvation.

When a population of microorganisms is unable to grow because the nutrients are either exhausted or cannot be used, mutants able to overcome the growth barrier are accumulating. These mutations are termed differently, but two of these terms, adaptive mutations and stationary phase mutations, are most widely in use (Foster, 1999a). Most of the studies on stationary phase mutagenesis have been carried out in defined laboratory environments where single parameters can be controlled more easily than in a complex natural environment. However, recent investigations indicate that similar processes that have led to genetic adaptation of microbial populations to a new environment in vitro can also take place in vivo, for example during the development of Pseudomonas aeruginosa biofilm in the lung of cystic fibrosis patients (Drenkard and...
et al. Cebula, 1992; Foster and Trimarchi, 1994; Rosenberg among mutants of actively growing bacteria (Prival and their specific spectrum that differs from that occurring characteristic features of stationary phase mutations is might control the mutagenic potential of cells. One of the own evolution, one may look for regulatory pathways that growth (Foster, 1999a).}

The vast majority of stationary phase mutagenesis studies have used starvation, either for a carbon source or for an amino acid (Foster, 1999a). However, the stationary phase mutation mechanisms may also be important in the development of antibiotic resistance mutations (Hall, 1998; Martinez and Baquero, 2000), colonization of new bacterial hosts (Giraud et al., 2001), phase variation in bacterial pathogens (Rosenberg, 2001) and may provide models for mutational escape of growth control, such as tumour progression (Strauss, 1992; 1998; Hall, 1995; Cairns, 1998).

The number of stationary phase mutations accumulating in a static cell population is higher than that expected theoretically if mutations occur at a normal growth-dependent mutation rate (Foster, 1999a). Some studies suggest that starvation conditions encountered during stationary phase incubation may permit a transient increase in the mutation rate because of a variety of factors, including decreased fidelity of DNA replication and reduction in DNA repair activity (Bridges, 1997; Foster, 1997; Sniegoski et al., 1997; Torkelson et al., 1997). Hence, if the rate of mutations can be elevated under conditions in which an excess of variation is most needed, i.e. during adaptation of a microbial population to a new environment, a question arises whether there are some mechanisms that have evolved to regulate the mutation rate in a cell.

Different mutagenic pathways in growing and in stationary phase cells

To evaluate the possibility that organisms can speed their own evolution, one may look for regulatory pathways that might control the mutagenic potential of cells. One of the characteristic features of stationary phase mutations is their specific spectrum that differs from that occurring among mutants of actively growing bacteria (Prival and Cebula, 1992; Foster and Trimarchi, 1994; Rosenberg et al., 1994; Kasak et al., 1997). These findings have suggested that stationary phase mutations occur via different molecular mechanisms from mutations that arise during growth (Foster, 1999a).

For over a decade, the most favoured system for the study of mutational processes in starving bacteria has been *Escherichia coli* strain FC40 (Cairns and Foster, 1991), which has a deletion of chromosomal lacZ gene and carries an engineered lac–lacZ fusion gene with +1 frameshift on F plasmid. Models to explain the mechanisms responsible for stationary phase mutagenesis in the FC40 system have been reviewed extensively (Foster, 1999a; 2000; Rosenberg, 2001; Hastings and Rosenberg, 2002). The mechanisms of mutability for Lac+ revertants in starving cells differ from mutability in growing cells. At first, the dissimilarity was found in different spectra of revertants (Foster and Trimarchi, 1994; Rosenberg et al., 1994). Mechanisms that are involved in the occurrence of stationary phase mutations using this test system include F− plasmid transfer functions, recombination, DNA repair and a special inducible error-prone DNA polymerase (pol IV) (Foster and Trimarchi, 1995; Longerich et al., 1995; Radicella et al., 1995; Foster et al., 1996; Harris et al., 1995; Rosenberg et al., 1996; McKenzie et al., 2001).

Alternatively, an amplification mutagenesis model was proposed by other investigators (Andersson et al., 1998; Hendrickson et al., 2002).

Another example of a regulated mutagenic pathway is resting organisms in structured environments (ROSE) mutagenesis that occurs only in bacterial colonies and involves composite control by two complex regulons: the SOS system and the catabolite repression system (Taddei et al., 1995).

Evidence supporting the idea that different mechanisms are responsible for the appearance of mutations in exponentially growing and stationary phase cells has also been found in *Pseudomonas*. The test system based on promoterless phenol degradation genes pheBA as a reporter enables the isolation and characterization of mutations accumulating in starving *Pseudomonas putida* cells (Kasak et al., 1997). The accumulation rate of Phe+ mutations on selective plates was found to depend on the physiological state of bacteria before plating; the accumulation was much higher for bacteria plated from a stationary phase culture than for those plated from a growing culture (Kasak et al., 1997). Moreover, the results of a recently published study (Saumaa et al., 2002) suggest that mutation processes in cells that have been starving for a short period are not entirely compatible with a prolonged starvation. It appeared that the spectrum of stationary phase mutations among early arising mutants differed from that of late arising ones. Although a particular C to A transversion was the dominant type of mutation during the first days of starvation, its share started to decrease later, and the percentage of other types of mutation, such as 2–3 bp deletions, increased. The occurrence of these deletions required stationary phase sigma factor RpoS (Saumaa et al., 2002).

Mechanisms of regulation of stationary phase mutations have also been studied in Gram-positive bacterium *Bacillus subtilis* (Sung and Yasbin, 2002). This report dem-
onstrated that accumulation of prototrophic revertants among \textit{B. subtilis} cells if placed under amino acid starvation pressure required the activity of at least two genes, comA and comK, which are known to regulate differentiation during post-exponential growth of \textit{B. subtilis}.

Cells exposed to a variety of DNA-damaging agents result in a dramatic increase in the mutation rate (Friedberg \textit{et al.}, 1995). \textit{E. coli} has five DNA polymerases. Three of these polymerases, pol II, pol IV, and pol V, are induced as part of the SOS regulon in response to DNA damage (Goodman, 2002). SOS induction has also been shown to occur spontaneously in static bacterial populations (Taddei \textit{et al.}, 1995). Thus, induction of error-prone DNA polymerases as a consequence of the SOS response in stationary phase cells would lead to mutagenic DNA synthesis and an increase in the number of mutations.

**Involvement of error-prone DNA polymerases in stationary phase mutations**

Recent data indicate that error-prone DNA polymerases may replicate significant stretches of DNA. The \(\beta\) subunit and the \(\gamma\) complex, although they were originally discovered as processivity subunits of pol III, increase the processivity of pol IV and pol V as well (Tang \textit{et al.}, 2000; Wagner \textit{et al.}, 2000; Maor-Shoshani and Livneh, 2002). Because the processivity mechanism is possibly shared by all DNA polymerases of a bacterial cell, this may offer a potential mechanism for DNA polymerases switching on DNA at sites where the replication fork collapsed at a blocking lesion (Sutton and Walker, 2001).

Most of the reversions of Lac\(^+\) frameshift mutations on F\(^+\) plasmid occurred via a DNA polymerase pol IV-dependent mechanism (Foster, 2000; McKenzie \textit{et al.}, 2001). The stationary phase mutations in the bacterial chromosome also required pol IV (Bull \textit{et al.}, 2001).

The F\(^+\) plasmid, which carries the lac allele, stimulates gene duplication and amplification (Hendrickson \textit{et al.}, 2002). F\(^+\) plasmid that has been used in a Lac\(^-\) reversion assay (plasmid F\(^*\)128) was formed by recombination between chromosomal sequences that placed the lac allele near the pol IV gene \textit{dinB}. Such a structure of the episome allowed co-amplification of \textit{dinB} with \textit{lac}, and an elevated mutation rate (Kofoid \textit{et al.}, 2003). Godoy \textit{et al.} (2000) observed that the absence of F\(^*\)128 plasmid resulted in a decreased rate of both accumulation of either plasmidial Lac\(^-\) or chromosomal Trp\(^-\) mutants and accumulation of additional mutations in Lac\(^-\) or Trp\(^-\) clones. Thus, it was concluded that some special feature of F\(^*\)128 (perhaps episomal location of \textit{dinB}) could be responsible for hypermutability. In the light of results from Kofoid \textit{et al.} (2003), it seems that overexpression of pol IV by amplification of the \textit{dinB} gene in the F\(^+\) plasmid could be one of the mechanisms that leads to general (genome-wide) mutagenesis studied by Godoy \textit{et al.} (2000).

The involvement of pol IV in stationary phase mutagenesis has also been shown in the case of other experimental systems. The expression of the ‘growth advantage in stationary phase’ (GASP) phenotype depends on the appearance of new mutations in the population (Zambreno \textit{et al.}, 1993; see also references in Finkel \textit{et al.}, 2000). Cells containing advantageous alleles permitting growth during stationary phase will increase their numbers relative to the rest of the population as the culture ages. They grow faster than parental cells using nutrients that become available from dying cells present in the culture. In the article by Yeiser \textit{et al.} (2002), the ability of SOS polymerase mutants to confer the GASP phenotype was examined. Mutant cells deficient in pol II, pol IV or pol V survived individually at least 2 months of stationary phase incubation, but they could not survive more than 10 days when co-cultivated with wild-type cells. Unlike wild-type cells that displayed a strong GASP phenotype in every case studied, the pol\(^-\) mutants in many cases either displayed a weaker GASP phenotype or were completely unable to compete with non-aged wild-type cells. These data indicate that all three alternative DNA polymerases (pol II, pol IV, pol V) are important for stationary phase mutagenesis. Thus, the enhanced accumulation of mutations resulting from specific properties of these three DNA polymerases confers a competitive fitness advantage to a subset of cells within the population during stationary phase. The GASP phenomenon is widely distributed. Several laboratories have observed the GASP phenotype in a wide range of microorganisms, including clinical isolates of \textit{E. coli} and other bacterial species (Turner \textit{et al.}, 2000; Kim \textit{et al.}, 2001; see also references in Finkel \textit{et al.}, 2000).

In addition to the above-described GASP mutants, a pol V-dependent mutagenesis pathway has been described for tryptophan-independent mutants that arose as a result of G:C to C:G transversions in a \textit{E. coli trpA} mutY tester strain starved for tryptophan (Timms \textit{et al.}, 1999). Data published so far have revealed that the stationary phase mutagenesis pathway that requires pol V occurs mostly in assaysing reversion of basepair substitutions, but not in test systems that measure frameshift reversions (Table 1). This difference appears clearly in the study by Bhamre \textit{et al.} (2001) in which Trp\(^+\) reversion frequencies of defined \textit{trpA} basepair substitution alleles and frameshift alleles were compared: the effect of \textit{umuC} deficiency on the reversion frequency was observed only in the case of basepair substitution alleles. Also, the \textit{umuDC}-encoded mutator DNA polymerase pol V was not required for stationary phase mutations at \textit{lac} frameshift on the F\(^+\) plasmid (McKenzie \textit{et al.}, 2001). These mutations, as well as chromosomal mutations studied by Bull \textit{et al.} (2001), were
frameshift mutations, and they occurred via a pol IV-dependent mechanism (see above). Nevertheless, other studies on growing cells have revealed that overexpression of pol IV can enhance both frameshift mutations and base substitutions (Kim et al., 1997; Wagner and Nohmi, 2000). Also, depending upon the nature of the DNA damage and its sequence context, pol V is able to induce either base substitutions or frameshift mutations (Napolitano et al., 2000). It is therefore likely that the mutagenic potential of pol IV and pol V in stationary phase mutagenesis is much broader than observed so far. However, one may also consider the possibility that the mode and timing of action of different polymerases can vary between growing and static cells.

**Efficiency of DNA repair systems in stationary phase cells**

As discussed above, error-prone DNA synthesis can increase the mutation rate in stationary phase cells. The efficiency of DNA repair in a cell is another factor that may control the frequency of mutations (Fig. 1). In many cases, the mutator phenotype is induced by inactivation of genes that code for DNA repair enzymes (Miller, 1998). The most potent mutator strains in *E. coli* are those lacking either the DNA polymerase III proofreading subunit (*mutD*) or the methyl-directed mismatch repair system (*mutS, mutL, mutH*). The *mutM mutY* double mutant of *E. coli* defective for DNA repair system that corrects mistakes occurring as a result of oxidation of guanine (GO repair) is just as strong a mutator as *mutD*, and about an order of magnitude stronger than a strain lacking *mutS* (Michaels et al., 1992). Therefore, it is tempting to speculate that DNA repair might be a limiting factor in stationary phase and nutritionally deprived cells, giving rise to stationary phase mutations. The next part of this section will discuss two prominent DNA repair systems in bacteria and analyse their efficiency in stationary phase cells.

To protect their genomes from oxidative DNA damage, bacterial cells have evolved efficient repair systems including MutY, MutM (Fpg) and MutT proteins (Michaels et al., 1992). Bridges et al. (1996) proposed that oxidized guanine residues, including 7,8-dihydro-8-oxoguanine (GO), constitute an important component of spontaneous mutation in stationary phase cells. MutT hydrolyses GO and depletes it from the nucleotide pool (Maki and Sekiguchi, 1992). GO is readily incorporated with equal frequency opposite C or A in a template strand (Maki and Sekiguchi, 1992). Also, when a GO lesion has occurred in DNA, DNA synthesis past GO can result in the misincorporation of adenine opposite the damaged guanine (Shibutani et al., 1991). The function of MutM glycosylase is to remove mutagenic GO from damaged DNA, and MutY glycosylase provides defense by removing A misincorporated opposite GO or G after DNA replication (Michaels et al., 1992). Cells that lack the activity of these glycosylases have elevated rates of G:C to T:A transversions (Nghiem et al., 1988). In *E. coli*, the accumulation of prototrophic mutants during amino acid starvation was caused by GO, and the rate of reversions enabling a prototrophic phenotype in starved cells was remarkably elevated in MutY-defective strains (Bridges et al., 1996).

**Table 1. Involvement of error-prone DNA polymerases in stationary phase mutagenesis.**

<table>
<thead>
<tr>
<th>Test system</th>
<th>Mutation type</th>
<th>Pol IV</th>
<th>Pol V</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac+ reversion on F’128 plasmid</td>
<td>–1 frameshift</td>
<td>+</td>
<td>–</td>
<td>McKenzie et al. (2001)</td>
</tr>
<tr>
<td>Lac+ reversion in <em>E. coli</em> chromos</td>
<td>–1 frameshift</td>
<td>+</td>
<td>–</td>
<td>Bull et al. (2001)</td>
</tr>
<tr>
<td>Trp+ reversion in <em>E. coli</em> chromos</td>
<td>Transversion</td>
<td>–</td>
<td>+</td>
<td>Bhamre et al. (2001)</td>
</tr>
<tr>
<td><em>E. coli</em> GASP mutants</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
<td>Yeiser et al. (2002)</td>
</tr>
<tr>
<td><em>E. coli</em> GASP mutants</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
<td>Yeiser et al. (2002)</td>
</tr>
</tbody>
</table>

![Fig. 1. Mechanisms that may disable DNA repair systems in stressed cells and elevate the mutation rate.](image-url)
Plasmids providing overproduction of MutY or MutM reduced the rate of mutation in starved cells (Bridges et al., 1996). The GO repair system is also an important defense system against the oxidatively damaged DNA in other organisms besides E. coli, for example in Pseudomonas (Oliver et al., 2002; Saumaa et al., 2002) and Deinococcus radiodurans (Li and Lu, 2001).

The methyl-directed mismatch repair (MMR) pathway corrects base-base and insertion–deletion mismatches that have escaped the proofreading function of replicative DNA polymerase or are produced by error-prone DNA polymerases (Harfe and Jinks-Robertson, 2000; Marti et al., 2002). MutS protein recognizes and binds to mismatches (Su and Modrich, 1986). In E. coli, together with MutH and in the presence of ATP, MutS activates a third MMR protein, MutH endonuclease (Grilley et al., 1989; Au et al., 1992). MutH cleaves the transiently unmethylated daughter strand at hemimethylated GATC sequences. In that way, MutH confers strand specificity on MMR as the newly replicated strand containing the error is targeted for repair. In starving E. coli, lac frameshift mutations in an F episome occur mostly as –1 deletions in single basepair repeats, whereas growth-dependent reversions are heterogeneous (Foster and Trimarchi, 1994; Rosenberg et al., 1994). Because these types of mutations are corrected by MMR, it led to the suggestion that the MMR pathway may malfunction in starved cells. The idea that MMR is disabled transiently during stationary phase mutagenesis was supported by the finding that the mutation spectrum observed in Lac+ revertants emerging in a starving cell population was reproduced by MMR deficiency in growing cells (Longerich et al., 1995). Subsequent studies by Feng et al. (1996) demonstrated that, when bacteria entered stationary phase, the amount of MutS in cells dropped at least 10-fold, and the amount of MutH dropped about threefold, whereas the amount of MutL remained unchanged. Unexpectedly, overproduction of MutL, but not of MutS, inhibited mutations in stationary phase cells carrying the lac frameshift reversion assay system on F′ plasmid, indicating that MutL somehow becomes limiting in stationary phase cells (Harris et al., 1997). The idea that stationary phase mutations may occur as a result of a decline in MMR has stimulated an active dispute. The commentary written by P. L. Foster (Foster, 1999b) argued that there is no valid evidence to support this idea. The questions raised in this commentary were addressed in another paper by Harris et al. (1999a).

Some other studies, originally proposed by Schaaper and Radman (1989), suggest that MMR deficiency is caused by saturation of the MMR system with an excess of DNA replication errors. Negishi et al. (2002) have demonstrated saturation of MMR using a mutagenic base analogue, and they have also shown that the introduction of a plasmid overproducing MutL was able to counteract the saturation. Wagner and Nohmi (2000) have shown that many pol IV-induced errors are corrected by MMR. Moreover, the results of this study revealed that errors made by a pol IV-titrated MMR enzyme MutL: artificial overexpression of MutL efficiently minimized the pol IV mutator effect in both wild-type and mutL backgrounds.

The MMR system can be a potent inhibitor of recombination between related species (Matic et al., 1996). In the human pathogen Streptococcus pneumoniae, the genetic plasticity is provided primarily by a natural transformation, whereas optimization of recombination is achieved by a transient inactivation of MMR and preinduction of recA in competent cells (Claverys et al., 2000). The S. pneumoniae MMR system Hex is unable to prevent interspecies transformation because the Hex system becomes saturated as a result of an excess of mismatches (Humbert et al., 1995).

A question about the evolution of evolution-accelerating systems

Most mutations are likely to be deleterious, and so the spontaneous mutation rate is generally held at a low level (Drake, 1991). However, a proportion of clones from natural populations of pathogenic and commensal bacteria has a strong mutator phenotype (LeClerc et al., 1996; Matic et al., 1997; Oliver et al., 2000; Richardson et al., 2002). Enrichment of spontaneously originated mutators in microbial populations undergoing adaptation to a new environment has also been shown in laboratory experiments (Sniegowski et al., 1997; Miller et al., 1999; Notley-McRobb et al., 2002a,b). By selecting a favourable allele, natural selection indirectly selects the sequence in which this mutation occurred. Consequently, mutator alleles can spread in microbial populations during adaptation by hitchhiking with the favourable alleles they produce: mutators can acquire favourable alleles more frequently than non-mutators and will therefore increase in frequency if the advantage of beneficial alleles is greater than the cost of being a mutator (Taddei et al., 1987). The appearance of a large number of mutators in populations undergoing adaptation to a new environment has led to the emergence of the concept of second-order selection (Tenaillon et al., 1999; 2001; Metzgar and Willis, 2000). According to this concept, indirect selection can act on mutation rates in the course of adaptation of a bacterial population: while selecting beneficial alleles, evolution indirectly selects for a system that creates these beneficial mutations.

Constitutive mutators might arise and persist only on a limited basis (de Visser et al., 1999; Notley-McRobb et al., 2002b). When passed through severe bottlenecks, mutator lineages also accumulate mutations that confer loss of fitness (Funchain et al., 2000; Giraud et al., 2001). To avoid a deleterious increase in global mutation rates

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that accompanies a mutator phenotype, some pathogenic bacteria (e.g. *Haemophilus influenzae*, *Helicobacter pylori*, *Neisseria meningitidis*) have evolved mechanisms for increasing the frequency of random variations in those genes that are critical in bacterium–host contact, nutrient acquisition and immune evasion (for a review, see Moxon *et al*., 1994; Henderson *et al*., 1999; Metzgar and Wills, 2000). Expression of the given genes (contingency genes) can be switched at a high frequency between functional (on) and non-functional (off) states by a mechanism known as phase variation (Henderson *et al*., 1999). Phase variation is usually associated with reversible mutations in homopolymeric tracts or tandemly iterated oligonucleotides in contingency genes. The replicative instability of repeat tracts can shift reading frames or alter promoter strength. The contingency loci are highly polymorphic in natural populations of *H. influenzae* and *N. meningitidis* (Bayliss *et al*., 2001). Results from De Bolle *et al.* (2000) have shown that mutation rates of a tetranucleotide repeat tract in the *H. influenzae* chromosome increase with the length of the tract. This indicates that the observed polymorphisms between strains in repeat tract lengths can alter mutation rates, raising the possibility that the rate of phase variation for individual contingency loci is itself under selection (De Bolle *et al*., 2000; Bayliss *et al*., 2001). Phase variation events have been presumed to be random with respect to their immediate selective value to the organism (Moxon *et al*., 1994). However, recent studies on bacterial adaptation to stress suggest that mutation rates at specific sites may be modulated by environmental conditions (Henderson *et al*., 1999; Massey and Buckling, 2002). For example, a phase-variable switching of the type 1 fimbrae in *E. coli*, necessary for colonizing the urinary tract, is sensitive to environmental factors such as temperature and nutrient availability, and is controlled by several DNA-binding global regulatory factors (reviewed by Henderson *et al*., 1999). Pseudomonads are known for their striking ability to adapt to various ecological niches: when selected in a spatially structured environment, bacterial populations diverge rapidly, producing a range of morphologically distinct niche specialists (Rainey and Travisano, 1998; Déziel *et al*., 2001). There are some recently published papers reporting the evidence that environmental signals can specifically control the rate of phenotypic switching in *Pseudomonas* species during pathogenesis and development of the biofilm (Sinha *et al*., 2000; Drenkard and Ausubel, 2002). Whether the loci responsible for phenotypic switching in pseudomonads can also contain mutational hot-spots is still unknown.

MMR not only protects against replication errors but also acts as a barrier to the recombination between moderately divergent DNA sequences (Matic *et al*., 1996). In enterobacteria, MMR-deficient cells carry out homologous recombination resulting from interspecies crosses three orders of magnitude more frequently than wild-type cells (Rayssiguier *et al*., 1989; Štambuk and Radman, 1999). Some authors have suggested that horizontal transfer and recombinational reshuffling have played an important role in the generation of microbial diversity (Ochman *et al*., 2000). As a consequence of the hyper-recombination phenotype, inactivation of the MMR gene increases the probability of reacquisition of functional a MMR gene by horizontal transfer. Comparative molecular phylogeny of MMR genes from natural *E. coli* gene trees demonstrated that, compared with the whole-genome trees, the MMR gene trees exhibit significant incongruence, explained as a result of horizontal gene transfer (Denamur *et al*., 2000). Similar observations were published by Brown *et al.* (2001). The authors of these articles conclude that MMR functions have been repeatedly lost and reacquired during the evolutionary history of *E. coli*. Recurrent losses and reacquisitions of MMR gene functions allow rapid generation of genetic diversity upon which selection acts during adaptation and restoration of a lower mutation rate after the adaptation. Rocha *et al.* (2002) hypothesized that inactivation of the MMR pathway might be positively selected. Their argument is based on the results of DNA sequence analysis indicating that mutS and mutL sequences, in comparison with 1000 random sets of genes, contain larger numbers of direct repeats potentially capable of inducing phenotypic variability by generating deletions. They also suggest that the results of the analysis are consistent with experimental evidence showing that deletions in the mutS gene are a major source of mutator phenotype in natural isolates.

Complex local mutator mechanisms also operate in eukaryotes: the best characterized example is mammalian antibody diversification by somatic hypermutation (SHM) (Harris *et al*., 1999b; Gearhart and Wood, 2001; Papavasiliou and Schatz, 2002). SHM of immunoglobulin variable (V) region genes is stimulated in B cells with antigens and results in the introduction of point mutations as well as occasional deletions and insertions at rates of 10^{-5}–10^{-3} per basepair per generation, about 10^{6}-fold above the normal level (Harris *et al*., 1999b). It was originally hypothesized that the substrate for somatic hypermutation might be a nick, a gap or a double-stranded break (Brenner and Milstein, 1966) that is repaired by an error-prone DNA polymerase (Gearhart and Bogenhagen, 1983). Recently published data support the DNA deamination model (Petersen-Mahrt *et al*., 2002), suggesting that the initial event in the somatic hypermutation is deamination of cytosines to uracils in DNA within the V region of immunoglobulin genes by an activation-induced cytidine deaminase AID (Di Noia and Neuberger, 2002; Harris *et al*., 2002; Petersen-Mahrt *et al*., 2002; Rada *et al*., 2002). The deaminated sites can be processed in several ways to generate mutations, whereas different replication
and repair pathways could be used to process AID-generated uracils (Gearhart, 2002). According to the model proposed by Gearhart (2002), high-fidelity DNA polymerases recognize uracil as thymine and pair it with adenine during the replication. Other mechanisms rely on the uracil first being removed by uracil glycosylase, leaving a gap. Low-fidelity polymerases will then bypass the gap during the replication or, alternatively, the action of these DNA polymerases may be combined with DNA repair pathways. There are indications that pol ζ, pol η and pol ι may be involved in somatic hypermutation (Diaz et al., 2001; Frank et al., 2001; Poltoratsky et al., 2001; Zeng et al., 2001; Faili et al., 2002; Pavlov et al., 2002).

Another potential mechanism for generating variation is environment-dependent induction of transient mutator phenotype (Radman, 1999; Metzgar and Wills, 2000; Radman et al., 2000). Here, the SOS-induced error-prone DNA polymerases (see above) are coming into a play. It is still an open question whether the error-prone DNA polymerases were selected during evolution for their ability to generate mutations or for their ability to allow cells to cope with damaged DNA. In the latter case, the reduced fidelity of these DNA polymerases may be the consequence of their specialization to replicate damaged DNA (Friedberg et al., 2002). Thus, are there any data supporting the idea that error-prone DNA synthesis itself may be positively selected? To look for such evidence, one should evaluate survival and fitness of cells in the presence and absence of mutator DNA polymerases.

Data published by Yeiser et al. (2002) demonstrate that, when compared with the wild-type cells, individually grown polymerase mutants exhibited an indistinguishable survival pattern. The reduction in fitness leading to the extinction of these mutants from the population became apparent only during their co-cultivation with wild-type cells under conditions in which the ability to generate GASP mutations was under selection. The authors suggest that the reduced fitness phenotype during the GASP competition cannot be wholly attributable to a reduced replication, but can be outweighed by the cells' ability to generate and maintain new advantageous alleles.

It has already been known for more than a decade that many plasmids confer an UV mutagenesis phenotype (reviewed by Woodgate and Sedgwick, 1992). UV mutagenesis, as we know, is associated with pol V functioning (see, e.g. Goodman, 2002). Here, it is important to note that there are many bacteria that do not contain functional analogues of the genes encoding pol V in their genomes. No homologues of umuD were found in the chromosomes of Gram-positive bacteria. However, the presence of functional analogues was assumed in their plasmids (Permina et al., 2002). Within Gram-negative bacteria, the genus Pseudomonas species examined so far lack chromosomally encoded pol V. Yet, many natural isolates of Pseudomonas strains contain plasmids that mediate UV mutagenesis (Kokjohn and Miller, 1994; McBeth and Hauer, 1996; Sundin et al., 1996). Although plasmid-encoded functions are not essential for survival of the host in some environmental conditions, they may well serve as such in many others. The presence of pol V genes in cells may be of importance only during genetic adaptation of microbial populations under changed environmental conditions.

Friedberg et al. (2002) have presented the concept of specialized DNA polymerases. In their review, they note that error-prone DNA polymerases are usually viewed as operating with low fidelity on normal template. If, instead, a particular lesion or class of lesions in DNA is considered to be the preferred template for specialized polymerases, these enzymes may be viewed as operating with high fidelity. Consequently, the specialized DNA polymerases are involved in mutation processes only when copying non-cognate DNA lesions or normal DNA. These enzymes are involved in mutagenesis in both prokaryotic (e.g. pol IV and pol V, as already discussed above) and eukaryotic cells (Friedberg et al., 2002; Goodman, 2002). In some cases, they participate in mutagenesis that is localized to specific sites: for example, recent evidence suggests that there could be at least two, and perhaps even more, error-prone (specialized) DNA polymerases involved in somatic hypermutation in immunoglobulin genes (Diaz et al., 2001; Frank et al., 2001; Poltoratsky et al., 2001; Zeng et al., 2001; Faili et al., 2002; Pavlov et al., 2002).
tion of error-prone DNA synthesis, should accumulate in order to saturate the DNA repair system leading to a hypermutable state of a cell (Fig. 1).

Under stressful environmental conditions, the movement of transposable elements may also increase the genetic diversity of microbial populations (Shapiro, 1997). An idea that transposition activity could be a response to challenges to the genome was originally suggested much earlier by McClintock (1984). In the late 1970s, transposable elements were described as parasitic sequences because they use the host machinery for their own amplification (Doolittle and Sapienza, 1980). However, an opinion increasingly expressed in recent years, suggesting that genome flexibility offers evolutionary benefits to an organism, may explain why a large number of transposable elements can be found all over the living world (Kidwell and Lisch, 2002). Studies of glucose-limited chemostat cultures have demonstrated that Tn5 and Tn10 can increase the fitness of E. coli (Biel and Hartl, 1983; Chao et al., 1983). The fitness effect of Tn10 was associated with transposition of IS10 into new sites in the genome (Chao et al., 1983; Chao and McBroom, 1985).

There is evidence that starvation is accompanied by increased transposition frequency of many mobile elements (Hall, 1988; Shapiro and Higgins, 1989; Mitter and Lenski, 1990; Thomas et al., 1992; Kasak et al., 1997; Lamrani et al., 1999). Naas et al. (1994) showed that a large number of IS-related rearrangements have occurred in a 30-year-old stab, leading to a highly polymorphic population of cells. Additionally, there are many transposable elements that can induce genomic variation in eukaryotic organisms (reviewed by Kidwell and Lisch, 2002). Recently published data suggesting the involvement of stress-activated transposition in plant evolution are based on the observation that, since the domestication of rice, an active transposon mPing has been amplified preferentially in cultivars adapted to environmental extremes (Jiang et al., 2003).

To avoid the potentially deleterious effect of transposition for host gene expression, the movement of mobile DNA elements is usually tightly downregulated. Increased transposition frequency may be the result of occasional inactivation of transposition control mechanisms, but it can also be a regulated process. So far, there are only a few published studies about regulation of transposition under stressful conditions. Transposition of Tn3-type transposon Tn4652 in P. putida is an exclusively stationary phase-specific event (Kasak et al., 1997), positively controlled by stress-specific factors IHF and stationary phase sigma factor RpoS (Ives et al., 2001). UV irradiation of E. coli cells stimulates intermolecular transposition of IS10, and this process is under the control of the SOS stress response (Eichenbaum and Livneh, 1998). Also, two growth phase-regulatory proteins (H-NS and RpoS) significantly alter the production of araB–lacZ fusions on Mu phage excision (Gomez-Gomez et al., 1997; Lamrani et al., 1999). Data obtained from these studies support the idea that (at least in the case of some mobile elements) activation of transposition under stressful conditions may be a regulated process, not merely malfunctioning of host defence mechanisms.

Many IS elements and transposons carry outward-directed promoters or part of promoter sequences (e.g. −35 hexamer) (Galas and Chandler, 1989; Mahillon and Chandler, 1998). These promoter sequences have no obvious functions related to the regulation of genes internal to the element. Therefore, as already originally proposed by Chao and McBroom (1985), the presence of outward-directed promoters at the ends of many transposable elements indicates that these elements have evolved to generate genetic variations.

**Concluding remarks**

Survival of microbial populations under stressful conditions is achieved using different strategies. Under a variety of growth-limiting conditions, bacterial cells express a general stress response and wait for better conditions. Only a few of them mutate. Yet, under conditions in which most of the cells in a population will die sooner or later if not adapted to a new environment, elevated mutation rates may be favoured in spite of a high cost paid by the generation of deleterious mutations. Later, when adaptation is achieved, a return to low mutation rates is selected for again. Several recent studies suggest that molecular mechanisms controlling mutational dynamics may have evolved to increase the rate of mutation when excess variation is most needed (Fig. 2). Evidence to support this hypothesis is becoming to accumulate.

There appears to be no single pathway responsible for stationary phase mutagenesis. Rather, a combination of different pathways seems to participate. So far, most of the research into stationary phase mutations has used E. coli model systems. Studies of mutational mechanisms in other bacteria have revealed that stationary phase mutagenesis is a general phenomenon in the microbial world. Ongoing investigations of this process using different model organisms and test systems will most likely reveal new mechanisms of mutagenesis.

It should be noted that stationary phase mutagenesis mechanisms have mostly been studied during short-term experiments, usually lasting only 7–10 days. However, in sharp contrast, a large majority of microorganisms in their natural environments face far longer periods of starvation. Therefore, to understand mechanisms of molecular evolution in microbial populations, more attention should be paid to stationary phase mutations occurring during prolonged starvation. Also, it would probably be more appro-
priate, from an evolutionary perspective, to envisage starvation as a quite natural metabolic situation for microorganisms, and to consider mechanisms to overcome it as a sophisticated list of different strategies that has been developed on its own right.

Acknowledgements

I wish to thank Richard Villems for useful criticism and comments on draft versions of this manuscript. I also thank laboratory members for suggestions. Work in the author's laboratory is supported by grants 4481 and 4482 from the Estonian Science Foundation, and by grant HHMI 5500316 from the Howard Hughes Medical Institute International Research Scholars Program.

Note added in proof

A recent paper by Bjedov et al. (2003) provides evidence supporting the hypothesis that stress-induced mutagenesis is a genetically programmed evolutionary strategy. This paper demonstrates that natural isolates of *E. coli* exhibit increased mutation rates under stress encountered in aging colonies. Mutagenesis in aging colonies (MAC) is associated with carbon-source starvation and oxidative stress, and is genetically controlled by RpoS and the carbon-sensing regulators CyaA and CRP.


References


Fig. 2. Genetic adaptation of microbial population under environmental stress: involvement of mutagenic pathways that may have evolved to elevate the mutation rate under conditions threatening the survival of microorganisms.


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