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Review

A new look at adaptive mutations in bacteria

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This is a short survey of the adaptive mutation processes that arise in non- or slowly-dividing bacterial cells and includes: (i) bacterial models in which adaptive mutations are studied; (ii) the mutagenic lesions from which these mutations derive; (iii) the influence of DNA repair processes on the spectrum of adaptive mutations. It is proposed that in starved cells, likely as during the MFD phenomenon, lesions in tRNA suppressor genes are preferentially repaired and no suppressor tRNAs are formed as a result of adaptive mutations. Perhaps the most provocative proposal is (iv) a hypothesis that the majority of adaptive mutations are selected in a pre-apoptotic state where the cells are either mutated, selected, and survive, or they die.

Adaptive mutations are spontaneous mutations that occur in bacteria after a prolonged period of incubation (3–7 days or longer) on non-lethal selective medium plates while the cells are not dividing or are dividing very slowly [1, 2]. They have attracted much interest because theirs specificity and spectrum of adaptive mutations, as well as their mechanism seems to be quite different from those arising in growing cells. The most intriguing aspect is that selectively advantageous mutations are more frequent than neutral mutations [3–6]. For example, when an *Escherichia coli trp*$^-$ *cys*$^-$ double mutant is incubated in the presence of cysteine, predominantly Trp$^+$, rather than Cys$^+$ revertants are obtained; and *vice versa*, when shortage of cysteine restricts growth, only Cys$^+$ but not Trp$^+$ revertants are seen. This phenomenon has also been observed for another pair of mutations [3, 7]. Thus adaptive mutations do not occur at random as in actively growing cells, but are selected to be advantageous.

Recently, non-selected mutations have also been observed during lactose selection in the FC40 system [8, 9], but their frequency ap-

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Abbreviations: EMS, ethylmethanesulphonate; MFD, mutation frequency decline; MMS, methylmethane sulphonate; TRCF, transcription repair coupling factor.

pears to be greater among the Lac^+ adaptive revertants than among the whole Lac^- populations. Thus, adaptive mutations seem to be associated with a transient state of hyper-mutation in starved bacteria [3, 4, 9]. Here we review the main features of adaptive mutations and propose hypotheses for their mechanism.

SYSTEMS TO STUDY ADAPTIVE MUTATIONS

Adaptive mutations are studied in experimental systems where the mutation whose reversion is tested is situated either on a plasmid or on the chromosome. $Lac^- \rightarrow Lac^+$ reversion in *E. coli* K12 strains has been most frequently studied, in which the mutant *lac* allele is situated on an F′ episome and the chromosomal lactose operon is deleted, namely, in SM195 (Δ*lac-pro*/F′$lacZ_{\text{amber}}$, pro^+), an amber *lacZ* mutant strain; and in FC40 (Δ*lac-pro*)/F′$lacI133_{+1}$-*lacZ*) a frameshift (+1 nucleotide) mutant in the *lacI* gene [1, 10, 11]. However, it was recently recognised that most late-arising Lac^+ revertants in SM195 cannot be classified as "adaptive" because they are, in fact, slow-growing suppressor revertants that probably pre-existed in the culture [12]. Furthermore, the Lac^+ revertants of FC40 strains are *recA* (but not *umuD*) and *recBCD* – recombination dependent, and rely on the presence of the plasmid *tra-* gene which encodes a protein that enables transfer of F′ episomes into bacterial cells. A further function is however necessary for adaptive mutations [13–15]. The characteristic of adaptive mutations of plasmid *lacZ* is that the reversion rate of $lac^- \rightarrow Lac^+$ is about 25–100-fold higher than when the same mutation is placed on the bacterial chromosome and is no longer *recA*-dependent [13, 14]. Thus, adaptive mutations of a gene situated on an F′ episome show features distinct from those occurring when the very same gene is situated on the chromosome. This may reflect the biology of the episome rather than that of the chromosome, and their mechanisms may be different [4, 12].

Adaptive mutation has also been studied in systems based on auxotrophy to prototrophy reversions of nonsense or missense mutations in the chromosome. For example $tyrA14_{ochre}$, $leu308_{\text{amber}}$ [16], *trpA23* (missense mutation at Arg_{211}, AGA instead of Gly-GGA; [17]), *trpE46* (unknown missense mutation [3]), $trpE65_{ochre}$ [18] in *E. coli* B, and K-12; and *hisG46* (missense) or $hisG428_{\text{ochre}}$ in *Salmonella typhimurium* [19], and the other systems.

THE SOURCE OF MUTAGENIC LESIONS AND THE ROLE OF DNA TURNOVER IN ADAPTIVE MUTATIONS

Several genes providing insight into the mechanism involved influence the frequency of adaptive mutations. A large increase in adaptive mutations was seen in bacteria defective in the *ada* and *ogt* genes, which encode alkyl transferases that remove O^6-alkyl-guanine and O^4-alkyl-thymine from DNA and thus protect from alkylation lesions. Therefore, increase in methylated bases leads to increase in adaptive mutations. The frequency of adaptive mutations was decreased in bacteria defective in *moa,* which codes for a molybdopterin cofactor and are hence deficient in nitrosation activity and shows reduced generation of a methylating agents [20] indicating on a role of nitrosation reactions in DNA alkylation.

A dramatic increase in adaptive mutations was also observed in bacteria defective in *mutY* or *mutT* genes, which are involved in protecting DNA from oxidative lesions [17, 18, 21–23].

MutY encodes a DNA glycosylase which excises A residues from A:G and A:8-oxo-G base pairs and thus protects from GC→TA transversions [24, 25]. 8-Oxo-dG in template DNA directs dCTP and dATP incorporation

and hence causes GC→TA transversions. Lack of MutY, and consequent mismatches in DNA, is most probably the source of small in frame deletions observed by Bridges & Timms [17] in the *trpA23* reversion system under starvation conditions.

MutT– encodes 8-oxo-dGTP pyrophosphatase that hydrolyses 8-oxo-dGTP to 8-oxo-dGMP and prevents it from being incorporated into DNA [26], and also possesses an activity, which degrades 8-oxo-rGTP in the ribonucleotide pool and hence prevents its incorporation into RNA and induction of a "leaky" phenotype [27, 28]. 8-oxo-dGTP is easily incorporated opposite an A residue in template DNA, but when it is in the template strand it predominantly directs dCTP incorporation and AT→CG transversions [29, 30].

A role of DNA, and possibly of RNA, turnover in adaptive mutation is clearly indicated by its increased frequency after damage to *mutT* [18, 28]. A role of DNA polymerases and hence of DNA turnover, is also indicated by works on the FC40 system of Foster *et al.* [31] and Harris *et al.* [32], and references therein. An antimutator allele of DNA polymerase III (*dnaE915*) reduces the frequency of adaptive mutations; mutator allele of DNA polymerase III (*dnaBex1*) increases the frequency of adaptive mutations.

That some metabolic activity in resting cells is essential for adaptive mutations to occur is evident from the observation that *tyrA14*→ $TyrA^+$ adaptive reversion does not occur in the *tas* mutant of WU3610 *tyrA14* [33]. *tas* encodes a protein with homology to the aldo-keto reductases that complement *tyrA14* dysfunction and allows considerable residual growth. Similarly a high residual growth under conditions of adaptive mutations that is observed in the FC40 $lacI^-$ strain is probably due to a very weak β-galactosidase activity encoded by the *ebgACB* genes [7] or in the other cases by impurities in agar [18]. Finally, slow growth of bacteria under starvation conditions may be made possible by programmed death of the neighbouring cells and reutilization of their nutrients, a process that must require some metabolic activity.

The above experiments indicate not only that the sources of the mutagenic lesions in resting cells seem to be the same as in growing cells (methylating and oxidating agents); but also that DNA turnover plays an essential role in the adaptive mutations.

MUTAGENIC SPECTRUM OF ADAPTIVE MUTATIONS

Adaptive mutations of *lac* +1 frameshift in the FC40 system arise almost exclusively by –1G nucleotide deletion in small runs of iterated nucleotides [11] in contrast to spontaneous reversions which usually occur by –1 or very large base pair deletions, or by +2 + 5 or more base pair insertions. Unexpectedly, Bridges & Timms [17] have found that during adaptive mutagenesis of missense mutations in the *trpA23* gene in a *mutT* and *mutY* background, a fraction of Trp^+ revertants arises as a result of different small (from 2 to 28 nucleotides) deletions that were not observed in growing cells. Such frameshift mutations in runs of small repeats are characteristic for DNA polymerase errors [34] consistent with an involvement of DNA polymerase and hence of DNA turnover in adaptive mutagenesis. Involvement of the *dam*-dependent mismatch repair system that may also lead to the small deletions is still under discussion [35], although the level of proteins involved in mismatch repair system is obviously decreased [36, 37]. Perhaps the strongest argument argued for a decrease in mismatch repair capacity under conditions of adaptive mutation is that spectrum of adaptive mutations and mutations in growing cells deficient in mismatch repair are the same [38].

No suppressor tRNA (extragenic) mutations were found under adaptive conditions in spontaneous His^+ revertants of *hisG46* (CCC, missense) and *hisG428* (TAA, nonsense) mutations in *S. typhimurium,* in contrast to those

occurring in growing cells [19]. Among the intragenic His^+ revertants arising by adaptive mutations at the *hisG428* site, there was an increase in the number of AT→TA transversions (by AAA, Lys codon formation) and a decrease in the number of AT to CG transversions (by TCA, Ser codon formation). It is also significant that no suppressor of tRNA was found among the Tyr^+ adaptive revertants of the *tyrA14* amber gene in *E. coli* W3610 [16].

RELATIONSHIP OF THE ADAPTIVE MUTATION TO THE MUTATION FREQUENCY DECLINE (MFD) PHENOMENON

The absence of tRNA suppressor mutations in adaptive revertants described above resembles the mutation frequency decline phenomenon (MFD). MFD is a rapid and irreversible loss of certain UV-induced mutations when UV-irradiated (and also ethylmethanesulphonate (EMS)- and methylmethanesulphonate (MMS)-treated) bacteria are transiently incubated under conditions in which synthesis of proteins does not occur [39–43]. Presently, we know that MFD is a special case of preferential repair of pre-mutagenic lesions from the transcribed strand of genes for tRNA, which requires a transcription repair coupling factor (TRCF) encoded by *mfd*, and UvrABC-endonuclease that excises a variety of modified bulky bases from DNA [44, 45]. In bacteria defective in either of these two genes the MFD effect for UV- and EMS-induced mutations do not occur [39, 41, 42] (although it is still seen for MMS [43]. Nothing is known about the MFD effect and repair processes in non-growing cells of *S. typhimurium*, but nevertheless, prolonged maintenance of cells under non-growing conditions may favour DNA repair and removal of pre-mutational lesions, perhaps preferentially from tRNA genes. Note that in SM195 strain, where the adaptive reversion is questionable, the allele of *lacZ* tested concerns an amber UAG triplet that is frequently reversed by tRNA suppressor formation. However, the frequency of adaptive mutations is about the same in *mfd*-deficient and *mfd*-proficient *E. coli* [46]. But this is not necessarily in contradictions with the preferential repair of tRNA genes. Since (i) an active *mfd* gene is not required for the MFD effect in MMS-treated cells [43], and most probably not for repair of oxidation lesions in DNA which are possibly the main cause of the adaptive mutations; and (ii) the period of starvation is much longer when adaptive mutations are tested than for MFD (days *versus* minutes), these are possible reasons why the role of the MFD protein may be reduced.

Based on these results, it may be suggested that the different spectra of spontaneous mutations in growing and in resting cells (adaptive mutations) are a reflection of the different activity of repair systems in growing and in resting conditions.

A HYPOTHESIS FOR THE MECHANISM OF ADAPTIVE MUTATIONS

It is most probable that the metabolism of starving cells differs distinctly from that of growing cells; protein synthesis is arrested, turnover of nucleic acids is low, and newly-synthesised DNA and RNA (if any) may be in an under-methylated state which may prolong period of action of the mismatch repair system. Limited growth is possible only when essential factors are furnished by leakiness of the transcribing or translating systems, expression of cryptic genes, and/or by lysis of the neighbouring cells. Both damage of DNA, due to accumulation of metabolites, and repair may be distinctly different than in growing cells and repair may resemble that occurring during the MFD phenomenon, when mutagenic lesions in potential tRNA suppressors are predominantly removed.

Glucose starved *E. coli* cells release dramatically increased levels of cAMP and cAMP-dependent SOS functions are induced [47] reflecting endogenous damage to DNA and arrest of DNA synthesis [48, 49]. In eukaryotic cells an increase in cAMP may lead to apoptosis, the programmed death of cells, which is also known to occur in bacteria infected by plasmids or viruses [50, 51]. The process of apoptosis is reversible for some period of time. It is possible that cells in a state of pre-apoptosis are most sensitive to DNA modifications and mutations, and when a premutation arises that allows by transcriptional leakness for resumption of growth, it may be expressed and fixed. All other non-selective mutations will be either not expressed and hence have no chance of fixation, or will be repaired and lost. Although damage to DNA occurs randomly in growing as well as in resting cells, the kinetics of DNA repair is distinct. Perhaps neutral mutations would have more chance to be detected when starved bacteria are briefly exposed to a rich medium to express non-selective mutations before being plated on the selective medium.

The mechanism proposed here in which a fraction of the starved cells becomes transiently hyper-mutable resembles in principle that suggested by others [3, 52, 9], but differs in the assumptions that the hyper-mutable fraction involves cells in a pre-apoptotic state and the bacterial cells will either mutate to release a selective stress and thus rescue growth, or will follow the programme of apoptosis and die. Alternatively, only those premutations whose phenotype allows for growth will be expressed; the remainder have more time to be subject to repair so that the majority of them will be lost.

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