Environmental regulation of mutation rates at specific sites

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Recent studies on bacterial adaptation to stress suggest that bacteria can regulate the generation of mutations at specific sites in response to environmental conditions. Here, we review these findings and discuss the circumstances under which these mechanisms might prove advantageous.

DOI: 10.1016/S0966-842X(02)02475-7

Pathogenic bacteria are exposed to continually changing, stressful environments such as transitions between different host environments, repeated exposure to antibiotics or attack by host immune systems. Such stresses impose strong selection for stress-resistant phenotypes, but it is unlikely that any single phenotype will be competitively superior in all environments [1]. Exposure to continuously changing, stressful environments might therefore indirectly impose selection for mechanisms that generate phenotypic variation, as such mechanisms could increase the probability of a successful phenotype being produced in a given environment [2–4]. Note that this selection will be indirect because genes responsible for variation-generating mechanisms will increase in frequency only when linked to a beneficial phenotype [2–4].

The type of mechanism for generating phenotypic variation that is favoured by selection is likely to be critically dependent on the range and repeatability of the environmental variation [1]. These mechanisms can be split into two categories: those that involve differential regulation of gene expression [5,6] and those that involve mutational events [7,8]. In this review, we focus on the latter group, specifically concentrating on recent work suggesting that the environment can regulate the rates of mutation at specific sites, resulting in variation of the rate of switching between a limited number of phenotypes, presumably as an adaptation to regular fluctuations between a fixed set of environments. Other mechanisms of phenotypic variation based on genetic variation, such as elevated genome-wide mutation rates [9], environmentally regulated genome-wide mutation rates [10] and elevated site-specific mutation rates [8,11], have been recently and extensively reviewed, and we mention these only briefly in the context of the environmental conditions in which they are likely to be favoured by selection.

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Elevated genome-wide mutation rates

The unexpectedly high frequencies of natural isolates of pathogenic bacteria showing elevated mutation rates as a result of impaired mutational machinery suggest there are natural conditions under which this trait confers an adaptive advantage [12–15]. Both theoretical and experimental work (*in vitro* and *in vivo*) suggests that mutator alleles that increase genome-wide mutation can increase the rate of adaptation to novel environments [16–19] (Fig. 1 illustrates how this can be beneficial). Although the majority of mutations generated will be neutral or deleterious, owing to their association with rare beneficial mutations the mutator alleles can be indirectly favoured by selection, and hence maintained in the population if continually exposed to novel environments.

Environmental regulation of mutation rates

The relative costs and benefits of elevated genome-wide mutation rates are likely to depend on the environment [20]. If bacteria are continually exposed to novel environments, selection might favour a high mutation rate. However, in non-stressful, relatively constant environments, the costs of producing deleterious mutations are likely to be greater than the benefits of producing rare, beneficial mutations (the costs and benefits of elevated mutation rates in bacteria are discussed in [20]). Pathogenic organisms are likely to be exposed to a range of environments, some more stressful, novel and variable than others, and it is possible that the ability to regulate mutation rates in response to environmental cues might be advantageous.

The existence of a stress-inducible hypermutation state has been proposed as a mechanism of regulating mutation rates, where stress (such as starvation) has been shown to trigger the SOS response [10,21–24]. The SOS system was originally identified as being a DNA repair mechanism that responds to physical DNA damage; induction of the SOS system activates error-prone DNA polymerases such as DinB (also known as PolIV) [25,26]. DinB is involved in copying bulky template adducts [27,28], but can also generate frameshift mutations [10]. In Fig. 2, the increase in the adaptability of an organism conferred by the ability to induce a hypermutational response to stress is illustrated.

Increased mutation rates at specific sites

Although elevated genome-wide mutation rates can prove advantageous, mutations at some sites are more likely to generate beneficial mutations than are

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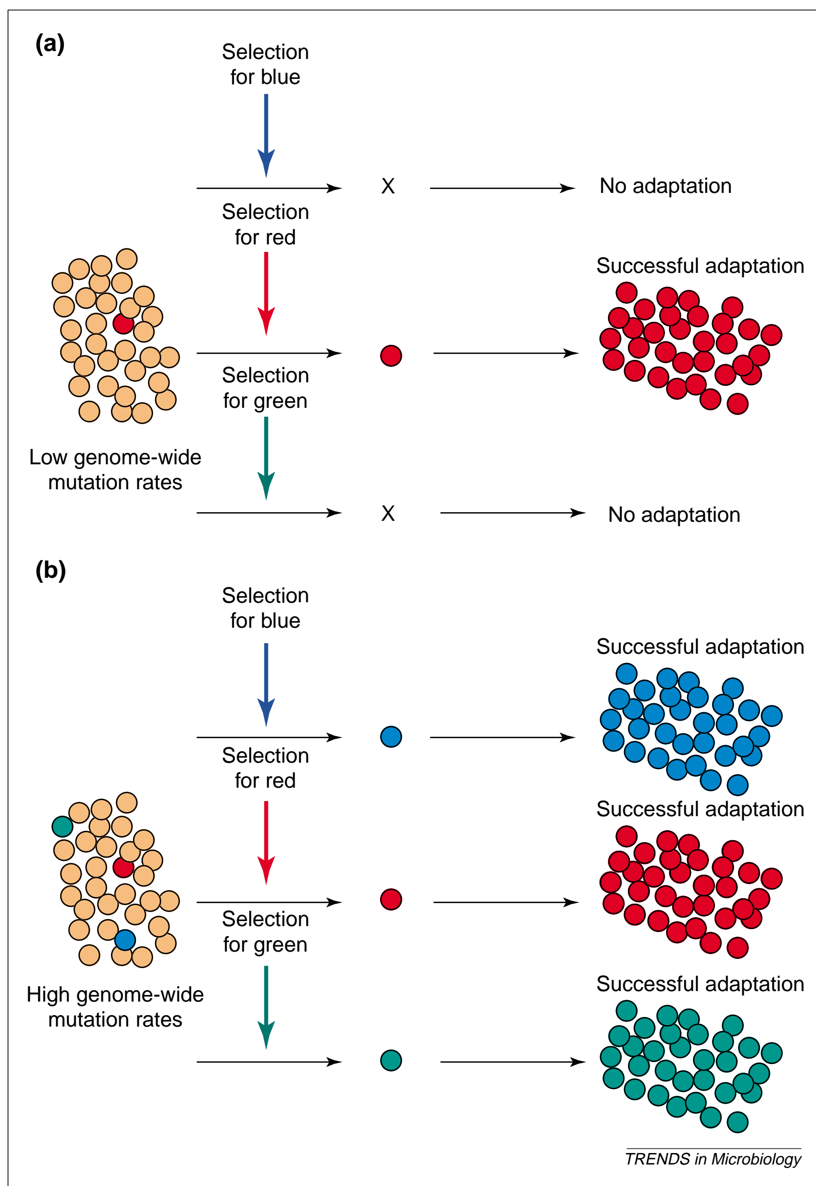

Fig. 1. Increasing the genome-wide mutation rates from (a) low to (b) high increases the variation upon which selection can act, facilitating adaptation to novel, stressful environment.

mutations at others [8]. For example, when under attack by immune systems, elevated mutation rates in genes encoding or affecting immunogenic proteins will generate a limited number of alternative phenotypes, one of which might provide a selective advantage by

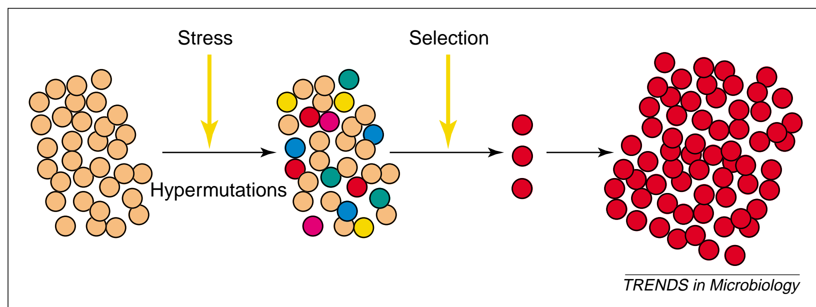

Fig. 2. Environmentally cued adjustment of mutation rates. An environmental signal such as a non-lethal stress can induce a hypermutational state that increases mutation rates, hence the probability of successful adaptation.

facilitating immune evasion. There would be no such advantage, and more likely a disadvantage, in mutating genes encoding proteins that do not affect what is exposed to the immune system. The host's immune system is a huge selective pressure for a pathogen, and thus the evolution of 'contingency loci', which increase the rate of mutagenesis at specific sites, confers an adaptive advantage by facilitating the generation of a limited number of variants [8] (Fig. 3). The mechanism used by bacteria to facilitate switching of contingency loci makes use of tracts of nucleotide repeats that are prone to undergoing strand slippage, resulting in either the disruption or establishment of a functional operator region [29] or the translational phase of a gene [30]. (See [9] for a recent review of these mechanisms.)

Environmental regulation of mutation rates at specific sites

Previous sections have discussed environmental control of mutation rates, and the ability to increase mutation rates at specific sites. The evolution of mechanisms that integrate these processes by enabling the environmental modulation of mutation rates at specific sites can confer considerable selective advantage. Such mechanisms can result in increased rates of switching between a limited number of phenotypes in response to environmental cues, and could possibly provide a selective advantage when there are frequent fluctuations between a limited number of known (previously encountered) environments (Fig. 4)

The clearest example of environmental control of mutation rates at a specific site is that of the phase-variable switching of the type 1 fimbriae in *Escherichia coli*. This involves recombination between repeat regions of DNA that causes the inversion of the promoter of the major subunit of the fimbriae, switching expression on and off [7,31]. The ability to switch fimbrial expression on and off is believed to confer a selective advantage by facilitating attachment to the host when fimbriae are expressed and, owing to their potentially immunogenic properties, evasion of the host's immune system when fimbrial expression is switched off. The rate and direction of switching of fimbriae is sensitive to environmental factors (temperature and nutrient availability), the effects of which can be mediated via DNA supercoiling and the global regulator HN-S [32–35]. How this sensing and responding is beneficial to the bacteria is not known in detail, but one can envisage a situation where the progression from one environment to another (e.g. from colonization of the urinary tract to the invasion of deeper tissue) might be associated with environmental changes that alter the fimbriate state of the cells. Perhaps with the future development of techniques with the sensitivity to detect levels of fimbrial expression *in vivo*, the biological relevance of such environmental control can be fully elucidated.

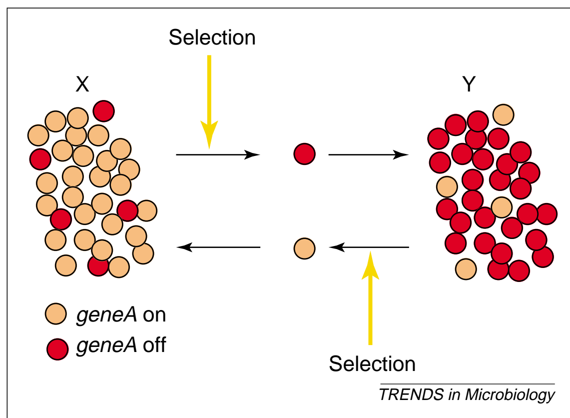

Fig. 3. Increasing mutation rates at specific sites: contingency loci. An organism is continually moving between environments X and Y, for example different concentrations of antibodies specific to particular immunogenic proteins. When *geneA* is on, the organism can replicate in environment X, but needs to switch *geneA* off to replicate in environment Y. Making *geneA* more mutable increases the probability of it being switched on and off and is therefore likely to be selected for by the organism continually moving between the two environments. This results in *geneA* becoming a contingency locus

Recent studies examining the enzymes involved in mutating contingency loci have revealed some interesting findings in relation to the potential role of the environment. Where the expression of a gene is to be switched on or off, the tracts of simple sequence repeats, or microsatellites, found within or near to the coding region vary in the nature of the repeats, and in the enzymes involved in their mutation. In *Haemophilus influenzae*, where di- and tetranucleotide repeats are used to vary expression of many virulence molecules, dinucleotide repeats are mutated by strand slippage controlled by the mismatch repair (MMR) system, and the mutation of tetranucleotide repeats is controlled by the DNA polymerase PolI [36]. In *Neisseria meningitidis*, mutation of the microsatellite resulting in the phase-variable expression of the haemoglobin receptor involves the MMR system [37], whereas phase-variable expression of the *siaD* gene, involved in lipopolysaccharide (LPS) biosynthesis, involves Dam methylation [38].

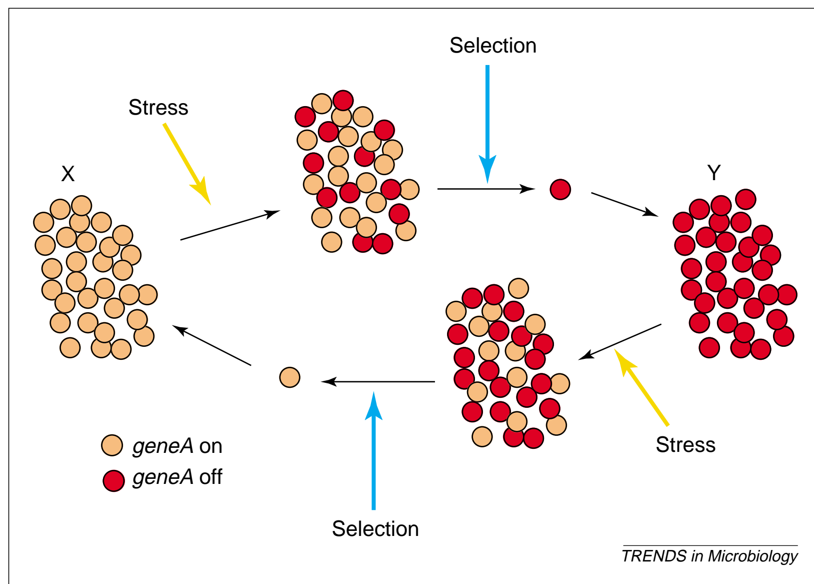

Fig. 4. Environmentally cued adjustment of mutation rates at specific sites. *geneA* is a contingency locus as described in Fig. 3. An environmental signal (such as differences in levels of osmolarity between environments X and Y) is always associated with moving between these environments and can be transduced to the bacterial cell via changes in the levels of DNA supercoiling. Connecting this signal to mutagenic apparatus that can then be directed towards the contingency locus allows the bacteria to adapt to changes in environment more rapidly, a trait likely to confer a fitness advantage.

Although a direct link between the environment and these mutagenic processes has yet to be demonstrated, the involvement of these enzymes suggests such links exist. Inactivation of PolI in *E. coli* is believed to induce the SOS response, increasing the generation of genome-wide mutations [39]; thus, environmental conditions that induce the SOS response (if present, or other stress-related systems in the absence of the SOS response) could affect phase variation of contingency loci. The MMR system and Dam methylation also respond to environmental stimuli. In *E. coli*, MMR and Dam methylation work together to control the direction of repair of mismatch mutations [25]. The methylation status of a piece of DNA will depend on its accessibility, as Dam cannot methylate DNA bound by protein. Global regulatory proteins such as OxyR (which responds to the oxidative status of the cell) bind DNA in response to environmental stimuli, and this binding can block DNA methylation and vice versa [40]. So, if DNA methylation and MMR are involved in controlling the rate of phase variation, and environmental cues control the level and sites of DNA methylation via regulatory proteins, then it is conceivable that the environment might be able to control the rate of switching at the contingency loci.

A stronger indication of a link between the environment and the mutation of contingency loci comes from a study of natural isolates of *Neisseria meningitidis* with mutator phenotypes that were found to have elevated rates of switching of the haemoglobin receptor [11]. Whether non-mutator strains of these bacteria can be forced by environmental factors into hypermutational states, and as a result of this modulate the rate of switching at contingency loci, merits future investigation. These future studies have the potential to provide a direct link between the environment and the mutation of specific sites.

The bacterium *Helicobacter pylori*, an important gastroduodenal pathogen of humans, has recently been reported to have the ability to switch phenotypically between lipopolysaccharide (LPS) structures in response to environmental pH [41], a mechanism believed to be mediated by strand slippage at contingency loci [42]. Successful colonization of the mammalian gut is dependent upon survival of exposure to pH gradient ranges of pH 2 to pH 7. In this study the bacteria were grown at pH 5 and pH 7, the structure of LPS analyzed and the conclusion drawn that pH was inducing a switch in LPS structure. Unfortunately, this study does not define the contribution of selection: is pH inducing the phase variation event as suggested or is phase

variation occurring equally in each environment, but the pH is selecting one LPS over the other? Experiments to clarify this are eagerly anticipated, and then the adaptive contribution of this sensing/switching ability can be investigated.

The final example we will discuss was brought to light when studying the ability of the bacterium *Staphylococcus aureus* to switch its resistance to an antibiotic on and off [43]. In this study the effect of induction was clearly separated from that of selection. The rate of emergence of resistant variants upon exposure to antibiotics was measured and, having counted the number of resistant variants in the inoculating population, the growth rate required for emergence of the resistant variants as a result of selection was estimated. However, this was not comparable with the actual growth rate of the resistant variant, demonstrating that the presence of the antibiotic was inducing the switch to resistance to occur.

When passaged in the absence of the antibiotic, sub-populations of these resistant variants begin to revert back to become antibiotic sensitive. Despite the reversibility of the switch, the observed heritability of both phenotypes suggests the event is occurring by a genetic switch. The ability to switch resistance on and off is adaptive because it allows the bacteria to overcome the negative fitness costs associated with permanent resistance [43]. The repeated exposure of this organism to the presence and absence of antibiotics (possibly following co-evolution with antibiotic-producing organisms) appears to have selected for not only a 'contingency locus' that allows it to switch resistance on and off, but also, indirectly, for a signalling mechanism that allows it to sense when it is advantageous to do so.

We do not suggest that the switches discussed in this section provide any evidence that the environment is influencing the direction of the switch. Rather, we suggest the repeated exposure to fluctuating environmental stresses has indirectly selected for the ability to modulate the rate and range of the generation of genetic variability. One or more of these phenotypic variants generated is likely to be advantageous under the environmental conditions that induce switching, and is thus selected for.

Conclusion

In this review we have discussed recent data suggesting that bacteria have evolved systems to respond to environmental cues by controlling mutation rates at specific sites. But one question that arises is why bacteria would evolve such mechanisms rather than mechanisms of phenotypic variation that do not involve genetic or mutational changes. One factor might be the speed of response to the environmental cue, but this is almost impossible to address owing to the confounding effect of selection by the environment following the adaptive change. Another possible reason might be that the relatively leaky nature (dependent upon concentrations of effector molecules within a cell) of non-mutation phase-variation mechanisms means that they would not be suitably efficient for use when providing a means to resist immediately lethal factors such as immune attack. Alternatively, there might be no greater reason for using one mechanism over another than that of which happened to evolve first.

The ability to adapt, by mutational mechanisms or otherwise, is central to bacterial pathogenicity. Studies on individual aspects of bacterial pathogenicity have provided us with invaluable information to aid in the battle against pathogenic bacteria but, perhaps in parallel with these studies, we should be concentrating on targeting the ability of bacteria to adapt. We do not believe 'adaptability' will be found to result from a single or simple process, but there is the possibility that common processes are involved. Combining measures that prevent the ability to adapt with currently effective treatments might contribute to preventing future adaptations by bacterial pathogens.

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