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Stress responses and genetic variation in bacteria

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Abstract

Under stressful conditions mechanisms that increase genetic variation can bestow a selective advantage. Bacteria have several stress responses that provide ways in which mutation rates can be increased. These include the SOS response, the general stress response, the heat-shock response, and the stringent response, all of which impact the regulation of error-prone polymerases. Adaptive mutation appears to be process by which cells can respond to selective pressure specifically by producing mutations. In *Escherichia coli* strain FC40 adaptive mutation involves the following inducible components: (i) a recombination pathway that generates mutations; (ii) a DNA polymerase that synthesizes error-containing DNA; and (iii) stress responses that regulate cellular processes. In addition, a subpopulation of cells enters into a state of hypermutation, giving rise to about 10% of the single mutants and virtually all of the mutants with multiple mutations. These bacterial responses have implications for the development of cancer and other genetic disorders in higher organisms.

Keywords

Spontaneous mutation; RpoS; Heat-shock; DNA polymerase; Error-prone DNA synthesis

1. Introduction

Because most mutations are detrimental, organisms have evolved mechanisms to keep their mutation rates as low as possible [1]. However, during adverse conditions, increasing genetic variation within a population could allow some members to achieve a phenotype that allows them to survive and proliferate. A transient increase in mutation rate would be particularly advantageous because then survivors would not continue to be burdened with a high mutation rate. Bacteria respond to stressful conditions by changing their patterns of gene expression so that the stress is relieved. These responses can be organized into more-or-less coherent pathways dealing with a particular stress, although there are clearly functions that overlap. Several of these stress response pathways include mechanisms that induce or enhance mutagenic processes.

2. The SOS response

When bacteria are subjected to DNA damage about 30 genes are coordinately induced, a reaction known as the “SOS response”. Induction of the SOS genes occurs when their common repressor, LexA, is inactivated. LexA inactivation is a proteolytic reaction that is greatly enhanced by RecA protein bound to single-stranded DNA. Obviously, the SOS response is highly induced after cells are exposed to DNA damaging agents, such as UV-light, that produce single-stranded DNA. But the SOS response is also induced, at least partially, whenever active LexA levels fluctuate downward. In vitro the rate of LexA inactivation increases when the pH

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becomes slightly alkaline [2]. In vivo LexA is inactivated when cells reach saturation in rich medium [3] and in aging colonies [4]. Thus, SOS genes may be induced to some degree under a variety of stressful conditions.

Recent years have seen the discovery of a hitherto unknown but widely distributed superfamily of error-prone DNA polymerases, the Y family (reviewed in [5]). Many of these polymerases can replicate damaged DNA, but this ability comes at the cost of frequent mutations on both damaged and undamaged templates. *E. coli* has two Y-family DNA polymerases, Pol IV, the product of the *dinB* gene (also called *dinP*) [6], and Pol V, the product of the *umuDC* operon [7–9]. Both polymerases are repressed by LexA and induced as part of the SOS response [10–12]. Pol V can replicate past a variety of DNA lesions; since the replicative DNA polymerase, Pol III, stalls at DNA lesions, Pol V has clear survival value for the cell. However, the ability of Pol IV to replicate damaged DNA is limited, and depends on the lesion and the sequence context (reviewed in [5]). Thus, the true role that Pol IV plays in the cell is not so clear.

The need to keep spontaneous mutation rates low under non-stressful conditions would appear to demand that the error-prone polymerases normally be tightly controlled. And, indeed, both the level and the activity of Pol V are controlled and targeted. In the absence of DNA damage there are only about 15 copies of Pol V in the cell (R. Woodgate, personal communication). In order to be active, the UmuDC operon must be induced (by LexA inactivation) and the UmuD protein must be cleaved to produce that active form, UmuD′. Both UmuD′ and UmuC are subject to degradation until they form the active Pol V complex, $UmuD'_2UmuC$. Even then, Pol V cannot replicate DNA unless RecA and single-stranded binding protein are present (reviewed in [5]). In contrast, Pol IV seems to be poorly controlled. In the absence of DNA damage, normal cells have about 250 copies of Pol IV enzyme [13]. Pol IV has no known cofactors. Even modest overproduction of Pol IV is a powerful mutator in growing cells [13, 14] and in stationary-phase cells (P.L. Foster, Submitted for publication). However, loss of Pol IV has little effect on normal, growth-dependent mutation rates, although this also depends on the mutational target [13,15,16] (P.L. Foster, Submitted for publication).

As LexA levels decline during starvation and other stressful conditions, the resulting induction of Pol IV can be expected to increase the error rate of any DNA synthesis that takes place. In non-proliferating cells, DNA synthesis may occur as a result of DNA repair or recombination. Several of the proteins required for recombination are also part of the SOS response, so levels of recombination may increase in stressed cells. The induced levels of Pol IV could allow it to out-compete more accurate polymerases for access to the DNA termini provided by recombination.

3. The general stress response

When bacteria enter the stationary phase of growth, a set of genes is induced by the activation of an alternative sigma factor, RpoS (σ^{38}), that directs RNA polymerase to their promoters. RpoS activity is also triggered by other stress conditions that have the common property of stopping growth. There are more than 70 genes in the RpoS regulon, and most of them encode proteins that help the cell survive the insults encountered by non-growing cells. Thus, RpoS is considered to be a master regulator of a general stress response (reviewed in [17]).

Recently we discovered that Pol IV is induced in late stationary-phase cells under positive control of RpoS; after induction, high levels of the protein are maintained for at least 3 days of continued starvation. This RpoS-dependent induction of Pol IV is independent of LexA inactivation [18]. Other researchers have shown that the *dinB* gene is transcribed in a 5-day-old culture [19]. Thus, in starving cells, Pol IV may reach levels that make it the dominant DNA polymerase, increasing the error rate of any DNA synthesis that takes place.

Mismatch repair is a crucial component of genomic integrity. The mismatch repair proteins survey newly replicated DNA and correct mismatches before they become mutations. Mismatch repair also inhibits recombination between diverged DNA, insuring that species integrity is maintained (reviewed in [20]). In *E. coli* key components of mismatch repair, particularly MutS (but not MutL), are down regulated in stationary-phase cells under control of RpoS [21,22]. Although mismatch repair is still active during starvation [23], certain cells in a starving population may have such low levels of the mismatch repair proteins that DNA polymerase errors are preserved. In addition, in cells without mismatch repair interspecies recombination would be enhanced, providing another source of genetic variation.

4. The heat-shock response

In *E. coli* heat-shock induces approximately 30 genes under control of another sigma factor, RpoH (σ^{32}). The RpoH-regulon is induced not only by temperature, but also by other conditions that result in unfolded proteins. In addition, certain components of the regulon, including GroE, are induced by DNA damage, oxidative stress, antibiotics and heavy metals, phage infection, and carbon source or amino acid starvation (reviewed in [24]). Thus the RpoH-regulon can also be considered a general-stress response. The *groEL/ES* operon, which encodes the molecular chaperone GroE, is an important member of this regulon. GroE is required at all temperatures to aid essential proteins to fold and maintain their proper conformation. Levels of DNA Pol V are dependent on GroE because the chaperon interacts with the polymerase subunit of Pol V and protects it from degradation [25]. Recently we found that GroE is also required for normal and induced levels of Pol IV. However, we could not detect an interaction between GroE and Pol IV; suggesting that GroE is required for the stability or activity of some other positive effector Pol IV (J.C. Layton, P.L. Foster, Submitted for publication).

5. The stringent response

Cells react to amino acid deprivation and other types of starvation by down-regulating the synthesis of stable RNAs (rRNA and tRNA). This response is mediated by an “alarmone”, guanosine tetra- (and penta-) phosphate, ppGpp. ppGpp alters RNA polymerase promoter selectivity so that the transcription of stable RNAs is decreased and the transcription of certain mRNAs is increased (reviewed in [26]). Starvation for a specific amino acid usually causes the genes for the biosynthesis of that amino acid to be derepressed, while the stringent response causes a general increase in transcription. Since active transcription tends to increase the mutation rate of the transcribed DNA [27–31], the stringent response provides a way that mutations can be directed to useful genes [30].

ppGpp is also a positive effector of RpoS and of RpoS-dependent genes [32,33]. Apparently ppGpp increases the ability of both RpoS and RpoH to compete with the vegetative sigma factor, RpoD (σ^{70}), for RNA polymerase [34]. Thus, the stringent response should enhance the induction of both the RpoS-dependent general stress response and the RpoH-dependent heat-shock response. As a result, the levels of Pol IV should increase and the levels of the mismatch repair proteins should decrease, resulting in a general mutagenic state.

6. Adaptive mutation

When populations of microorganisms are subjected to non-lethal selection, mutations arise that relieve the selective pressure [35]. This phenomenon, originally called “directed mutation”, is now called “adaptive mutation”, by which is meant a process that produces advantageous mutations during selection even though other, non-selected, mutations occur at the same time.

6.1. Adaptive mutation in a Lac⁻ strain of Escherichia coli

Adaptive mutation has been most thoroughly studied in *E. coli* strain FC40 [36]. This strain is Lac^- but reverts to Lac^+ at a high rate when lactose is its only carbon and energy source. FC40 has a +1 frameshift mutation in the *lacI* coding region that affects the *lacZ* gene, making the strain Lac^- [37]. The *lac* region as well as *proAB*$^+$ are carried on a conjugal plasmid, F′128, and deleted from the chromosome.

When FC40 cells are plated on minimal lactose plates, the first crop of Lac^+ colonies appears after 2 days (the time it takes a Lac^+ cell to produce a colony). If independent cultures are plated in parallel, the numbers of mutants among the cultures have a Luria Delbrück distribution, meaning that the Lac^+ mutations occurred while the cells were growing prior to plating. These growth-dependent mutations occur at a rate of about 10^{-9} Lac^+ revertants per cell per generation, which is a normal rate for reversion of a point mutation. With continued incubation, Lac^+ colonies accumulate and the distribution of these mutants among cultures is Poisson, meaning that mutations occurred after plating. These are the adaptive mutations, and they occur at a rate of about 10^{-9} per cell per hour. After 5 days on lactose plates, there are about 100–200 Lac^+ colonies per 10^8 cells plated [36].

Unreverted cells of FC40 cannot proliferate on lactose. When a population of Lac^- FC40 cells are incubated in liquid lactose medium, no increase in turbidity or in cell number can be detected for 3 days [36,38]. In addition, there is no detectible increase in the amount of *lac* DNA (relative to house-keeping gene) [39]. These results mean that the majority of Lac^+ adaptive mutations that appear during a 5-day experiment are arising in a population that is neither growing nor amplifying its *lac* DNA. However, when incubated on minimal lactose agar plates, the Lac^- population can grow on impurities in the agar. This growth can be controlled by adding "scavenger cells" that are Lac^- but cannot revert to Lac^+. In the presence of 10-fold excess scavengers, the FC40 Lac^- population is stable on minimal lactose plates for about 5 days [36,38].

6.2. Adaptive mutations are not directed

In their original 1988 paper, Cairns et al. hypothesized that mutations could be "directed" toward a useful goal [35]. However, the original evidence supporting this hypothesis has not survived subsequent study. The first negative evidence was obtained not with FC40, but with SM195, a strain with an amber mutation in *lacZ* [35]. SM195 reverts both by true reversion and by the creation of extragenic tRNA suppressors, and both classes of revertants appear continuously during lactose selection. Because in the case of extragenic suppressors there is no direct path from the phenotype (Lac^+) to the mutated gene (encoding a tRNA), the hypothesis that the selective conditions "instructed" the cell to make appropriate mutations could be dismissed [40]. It was later shown that about 2/3 of the late-appearing suppressors of SM195 were slow-growing and probably arose during growth prior to lactose selection [41]. Nonetheless, the continuous appearance of fast-growing suppressors demonstrates that mutations occur elsewhere than in the gene under selection [42].

The directed mutation hypothesis predicts that mutations that are not selected do not appear. This had appeared to be true when irrelevant genes, *ilvG* and *rpoB*, were monitored for mutation during lactose selection [35,38]. However, the target for Lac^+ reversion is on F, the conjugal plasmid, and mutation of genes on F appears to occur at a higher rate and by a different mechanism than mutation of genes on the chromosome [16,43,44]. When a second revertible allele, a +1 frameshift in the *tetA* gene, was on the episome close to the Lac^- allele, TetR revertants appeared at about the same rate as did Lac^+ mutations during lactose selection. In addition, TetR reversion had the same genetic requirements as mutation to Lac^+ [45]. Although the two mutational events were probably not independent [45], these results demonstrate that

the mutational mechanism that gives rise to adaptive mutations is not directed to the gene under selection.

6.3. The genetics of adaptive mutation

The mechanism of adaptive Lac^+ mutation in FC40 has been recently reviewed [46]. The following is a summary of the relevant characteristics:

- **i.** The DNA sequence changes that give rise to adaptive Lac^+ mutations and growth-dependent Lac^+ mutations are different. Adaptive Lac^+ mutations are almost all −1 bp frameshifts in runs of iterated bases [47,48], whereas growth-dependent mutations include deletions, duplications, and other frameshifts.
- **ii.** The recombination functions for double-strand break repair are required for adaptive but not for growth-dependent Lac^+ mutation. The required genes are *recA*, *recBCD*, *ruvAB*, and *ruvC* [36,49–52]. In contrast, mutations in *recG*, which encodes an alternative recombination resolution function, increase the rate of adaptive mutation dramatically [51,52]. This is mostly, or entirely, due to the fact that error-prone Pol IV is overexpressed in *recG* mutant cells [18].
- **iii.** The high rate of adaptive mutation to Lac^+ requires that the Lac^- allele be on the F plasmid; when the same Lac^- allele is at its normal position on the chromosome, the rate of adaptive mutation falls about 100-fold [43,53] and the mutations are not recombination-dependent [43].
- **iv.** The high rate of adaptive mutation to Lac^+ requires that conjugal functions be expressed [43,54], although actual conjugation is not required [43,55]. The rate of adaptive mutation falls 10-fold when conjugal functions are defective, but the mechanism by which the Lac^+ mutations occur is still recombination-dependent [43]. The role of the conjugal functions is most likely to stimulate recombination by producing DNA nicks at the conjugal origin [56].
- **v.** The rate of adaptive mutation is reduced two- to four-fold if Pol IV is eliminated [57,58]. *E. coli*'s other inducible error-prone polymerase, Pol V, is not involved in adaptive mutation [36,59].
- **vi.** About 90% adaptive Lac^+ mutations are eliminated if the general-stress sigma factor, RpoS, is mutant [18,60]. Part, but not all, of this reduction is because RpoS is a positive effector of Pol IV [18].

6.4. Model for adaptive Lac^+ mutation

Our current model for adaptive mutation to Lac^+ is as follows [46]. When FC40 is incubating on lactose, the cells are not proliferating but replication is occasionally initiated at one of the episome's vegetative origins. Nicking at the conjugal origin is persistent in starving cells [61]; when the moving replication fork encounters this nick, the fork will have a high probability of collapsing, creating a double-strand end. Double-strand break repair is then initiated by RecBCD. When RecA catalyzes the invasion of a single-strand into a homologous duplex, new DNA synthesis is primed from the 3′ end. If Pol IV does this synthesis, mutations result. Eventually a new replication fork incorporating the normal DNA polymerase, Pol III, is established. The four-stranded recombination intermediate is then resolved by RuvAB-catalyzed branch migration and RuvC-catalyzed strand cleavage.

7. Hypermutation

When Lac^+ mutants that arise during lactose selection are isolated and assayed for other phenotypes, about 1% prove to have second mutations somewhere in their genome [62–65].

Yet these isolates do not prove to have a high mutation rate upon subsequent testing [62,63]. These results strongly suggest that during lactose selection a subpopulation of cells has undergone a period during which their mutation rate was transiently elevated. Transient hypermutation was predicted by Hall [66] and modeled by Ninio [67] and Cairns [63,68].

In *E. coli* strain FC40 hypermutation requires Pol IV [69]; in addition, mismatch repair is not active among hypermutators [63]. Thus, the hypermutator state appears to be due to the combination of induced expression of Pol IV plus decreased activity of mismatch repair in a small subpopulation of cells [69,63]. About 0.1% of the population are hypermutators and their mutation rate is elevated about 200-fold. The hypermutators produce about 10% of the Lac^+ adaptive mutations, whereas 90% of the Lac^+ mutations arise in "normal" cells by the pathway outlined above. However, virtually all cases of multiple mutations arise in hypermutators [63].

8. Summary and significance

Recent evidence strongly suggests that both adaptive mutation and hypermutation are induced responses to stress. Many of the functions required – RecA, Pol IV, and RuvAB – are induced as part of the SOS response to DNA damage. The SOS response is also induced in aging colonies [4] and at the end of growth in rich medium [3]. In addition, Pol IV is positively regulated by the general-stress sigma factor RpoS [18], and is expressed in starving cells [18, 19]. Key components of mismatch repair are down regulated under control of RpoS [22]. Although mismatch repair is active during lactose selection [40], the fact that mismatch repair proteins are in low supply may mean that in some cells the pathway is saturated, or some components are not present (as suggested by Ninio [67]), giving rise to the hypermutator population.

There are several aspects of adaptive mutation in FC40 that may be important in evolution. The first is recombination-dependent mutation. In our strains this mechanism is particularly active on the F′ element that carries the *lac* allele, probably because of the persistent nick induced at the conjugal origin. But the same mechanism is expected to occur whenever a nick is encountered during DNA replication. This may not be a major source of variation in proliferating cells when other mutational mechanisms are active, but it might become significant in static populations.

A number of experimental and theoretical studies have shown that individuals with high mutation rates can have a selective advantage in changing environments [70–72]. Indeed, models predict that the random appearance of a mutator allele can accelerate the adaptive evolution of an entire population [73]. A transient mutator state would be even more advantageous. When confronted with an adverse situation, only a very small proportion of the population would become hypermutators. If the current problem can be solved with a single advantageous mutation, it is likely to appear not in the hypermutators, but in the normal cells. As the successful cells proliferate, they would carry no extra mutational burden. However, if advantageous mutations are rare, or if more than one mutation is needed, the hypermutating cells would succeed and proliferate. They would carry extra mutations, but because the hypermutable state is transient, their mutation rates would return to normal, minimizing the genetic burden carried by their progeny.

The Y-family of error-prone polymerases is found in all three domains of life. Eucaryotes have a plethora of these polymerases, each of which appears to be more-or-less specialized to replicate pass particular DNA lesions. On both damaged and undamaged DNA, some of the eucaryotic polymerases have stunning error rates. For example, the error rate of DNA polymerase ι can be as high as 1% [5,74]. Because mutations are rare in normal somatic cells,

but tumors have multiple mutations and display profound genetic instability, it was hypothesized many years ago that a mutation giving a mutator phenotype would be one of the early steps in carcinogenesis [75]. One class of such mutator mutations could be loss of cell functions that control or target the activity of an error-prone polymerase. Alternatively, a transient mutator state could result from the induction of an error-prone polymerase as part of a stress response. Recently both breast and lung cancer cells have been shown to have elevated levels of an error-prone polymerase [76,77]; in the breast cancer cells, Pol ι was further induced by exposure to UV-light [77]. Further research into the mechanisms that regulate the activity and the error-prone polymerases will shed light on the role that these enzymes play in the development of cancer and other genetic diseases.

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