EVOLVING RESPONSIVELY: ADAPTIVE MUTATION

Susan M. Rosenberg

A basic principle of genetics is that the likelihood that a particular mutation occurs is independent of its phenotypic consequences. The concept of adaptive mutation seemed to challenge this principle with the discoveries of mutations stimulated by stress, some of which allow adaptation to the stress. The emerging mechanisms of adaptive genetic change cast evolution, development and heredity into a new perspective, indicating new models for the genetic changes that fuel these processes.

> "The Haggunenons of Vicissitus Three have the most impatient chromosomes of any life form in the Galaxy. Whereas most races are content to evolve slowly and carefully over thousands of generations, discarding a prehensile toe here, nervously hazarding another nostril there, the Haggunenons would do for Charles Darwin what a squadron of Arcturan stunt apples would have done for Sir Isaac Newton. Their genetic structure, based on the quadruple sterated octohelix, is so chronically unstable, that far from passing their basic shape onto their children, they quite frequently evolve several times over lunch. But they do this with such reckless abandon that if, sitting at table, they are unable to reach a coffee spoon, they are liable without a moment's consideration to mutate into something with far longer arms … but which is probably quite incapable of drinking coffee."
>
> *Douglas Adams*
> *The Hitch-hiker's Guide to the Galaxy*[1]

Departments of Molecular and Human Genetics, Biochemistry, and Molecular Virology and Microbiology, Baylor College of Medicine, Room S809A, Mail Stop 225, 1 Baylor Plaza, Houston, Texas 77030-3411, USA.
e-mail: smr@bcm.tmc.edu

Douglas Adams' conception of the Haggunenons was supposed not to resemble the real processes of genetic change that lead to biological evolution. It is biology (deliciously) askew because it merges two components of the process of evolution that, in the neoDarwinist view (for example, REF. 2), are regarded as being independent: the generation of heritable variation (for example, mutation) and natural selection (by the environment). Less than a decade after Adams' play was aired, life seemed to imitate art. Reports appeared of various instances of 'adaptive' mutation (BOX 1), whereby genetic variation apparently occurs in response to the environment, rather than independently of it. Before this, spontaneous mutation was thought to occur exclusively under defined circumstances: in growing cells (usually measured as mutations per cell per generation); before the cell encounters an environment in which the mutation might prove useful; and in any gene, irrespective of the usefulness of the mutation (for example, REFS 3–5). By contrast, Shapiro[6], followed by Cairns *et al.*[7], in their studies of adaptive mutations in *Escherichia coli*, reported mutations that occurred: in non-growing or slowly growing cells; after the cells

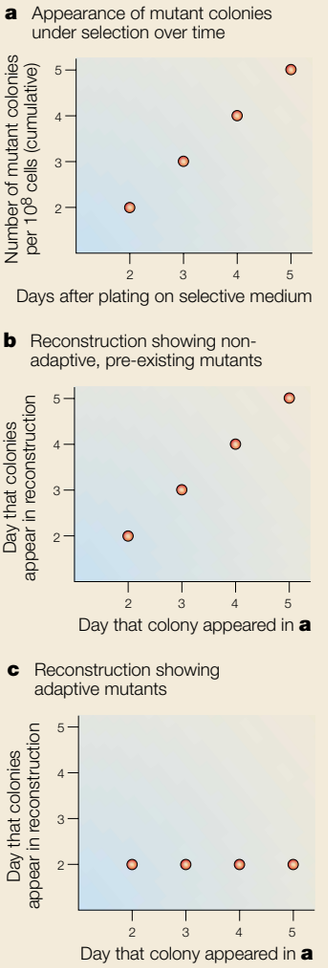
Box 1 | Defining adaptive mutation

The term 'adaptive' mutation was used by Delbrück[118] to indicate mutations formed in response to an environment in which the mutations were selected. The term does not imply that non-adaptive (unselected) mutations would not also be induced, or that the useful mutations would be induced preferentially (this latter idea is called 'directed' mutation). 'Adaptive mutation' was adopted subsequently by Tlsty in her examination of gene amplification in rat cells[93]. She distinguished[3,93] mutations that pre-exist at the time a cell is exposed to a selective environment versus (adaptive) mutations formed after exposure to the environment. Cairns and Foster[12] have used the term similarly. 'Adaptive mutation' is used in this sense here and not to indicate any useful mutation formed at any time.

Box 2 | **Criteria for determining adaptive mutations**

Experimental proof of 'adaptiveness' requires a demonstration that the mutations in question formed after cells encountered the growth-limiting environment in which the mutations allow growth. There are several ways to show the opposite: mutations that are formed during growth of a culture before exposure to a selective environment[3–5]. The most famous of these, the fluctuation test of Luria and Delbrück[3], involves the analysis of the distribution in the numbers of mutants that arise in each of multiple replicate cultures of cells under selection. If mutations form before selection, the numbers of mutants in the multiple cultures yield a particular, highly variable distribution[3]. However, if fluctuation tests do not give the Luria–Delbrück (variable) distribution, further evidence is needed to assess whether or not the mutants were formed after encountering the selective environment (for example, REF. 119) (that is, adaptively). This can be tested using 'reconstruction' experiments. A prototypical example is the *Escherichia coli lac* frameshift reversion assay[12]. This assay uses *E. coli* cells that are deleted for the chromosomal *lac* operon[12], which encodes enzymes required to metabolize lactose, but that carry a *lac* +1 frameshift mutation on an F′ conjugative plasmid. Cells that revert the frameshift mutation are easily detected as they form growing colonies on lactose-minimal medium. In the assay, about 10^8 Lac^- cells are spread onto a lactose-minimal medium plate and are incubated for several days, during which new Lac^+ mutant colonies appear (a**); by contrast, the 10^8 Lac^- cells do not increase in number (FIG. 1a)[12]. Mutant colonies that appear late (on day 5, for instance), do not show a Luria–Delbrück distribution in multiple cultures[12]. However, they could still result either from mutations that occur after plating on lactose (that is, adaptively) giving mutants with normal growth rate, or they could result from slow-growing, non-adaptive, mutant cells that were present before plating on lactose. To distinguish between these two possibilities, mutant clones are isolated from an experiment such as in (**a**), and a known number of cells of each mutant isolate (for example, 100) is plated in a reconstruction of the conditions in the original experiment: that is, on lactose medium in the presence of 10^8–10^9 Lac^- neighbour cells. If cells from the original day 5 colonies (from **a**) reform colonies that appear five days after plating (**b**), then the mutations are inferred to have formed before exposure to lactose medium, and therefore, non-adaptively. If the resulting colonies appear sooner than day 5 (**c**), the original mutation is inferred to have occurred adaptively.**

In the *lac* frameshift reversion assay[12], both POINT MUTANTS[36] and amplified isolates[13] were shown to be genuinely adaptive by this reconstruction test. A test was used to show that, among the original, presumed adaptive mutants of Cairns *et al.*[7], most reversions of an *E. coli lac* amber allele were really pre-existing (non-adaptive) mutants[11]. (A similar test in the original paper[7], but omitting the Lac^- neighbour cells, gave the opposite result.) The possibility that the minority were growth-dependent mutants that formed during growth on the lactose plates has not been ruled out in that system[11].



were exposed to conditions that favour the mutant; and, they suggested[7], preferentially in those genes that could allow growth if mutated. (For antecedents, see REFS 8–10.) This last suggestion was provocative in its implication that cells might acquire advantageous mutations specifically. In their study, Cairns *et al.* assayed for the reversion of an AMBER MUTATION in the lactose catabolism (*lac*) operon of *E. coli* cells. Revertant cells were scored by their ability to grow on lactose-minimal medium. Most of the mutations in question[7] were not directed to specific genes or even formed adaptively[11] (BOX 2). However, in a second assay published by Cairns and Foster[12], which measures reversion of a *lac* frameshift (rather than a nonsense) allele carried on an F′ CONJUGATIVE PLASMID in *E. coli* that are starving on lactose-minimal medium, the real thing occurs. Mutations occur by mechanisms unlike spontaneous, growth-dependent mutation and adapt the cells to their environment. The result is similar to Adams' "far longer arms". However, the mutations are not directed to the gene in which mutations could be advantageous, as unselected mutations also accumulate (perhaps like becoming "quite incapable of drinking coffee").

Two mechanisms of adaptive genetic change are now known to occur in the *lac* frameshift system: adaptive point mutation and newly shown (although previously postulated) adaptive gene amplification (REF. 13 and references therein for earlier work). These are the best understood of any adaptive mutation mechanisms and are the main focus of this review. There are many other bacterial (for example, REFS 14–25) and yeast[26–32] assay systems in which adaptive and stationary-phase mutations have been reported, but about which much less is known. In one assay, the mutations might be directed to genes under selection[25]. But for most of the assays, with important exceptions[17,20,32–34], information about the mutation mechanisms is lacking and it is thus unclear whether they differ from growth-dependent mutation.

The adaptive genetic change mechanisms in the *lac* frameshift system of *E. coli* look like inducible genetic chaos, from which the metaphorical coffee drinkers emerge and thrive. The point mutation mechanism includes the following components: DNA breakage; recombinational break repair; transient mismatch repair limitation; genome-wide hypermutation in a subpopulation of cells that gives rise to some or all of

AMBER MUTATION
A mutation due to the introduction of a stop codon (UAG) in the coding sequence of a gene that results in premature termination of translation.

POINT MUTATIONS
Small changes to DNA, such as base substitutions, small deletion and insertion (frameshift) mutations, made in the context of a gene. They are contrasted here with large-scale genome rearrangements such as gene amplifications and large deletions, inversions, translocations and chromosomal instability.

Box 3 | **Processes involved in DNA repair and mutation**

Mismatch repair
A DNA repair process conserved in eubacteria and eukaryotes that recognizes mispaired bases caused by replication errors, for example, and corrects them in favour of the old DNA strand. Mismatch repair processes correct both substitution mismatches (for example, A paired with G) and small insertion/deletion (1–4 nucleotide) loops.

Recombinational break repair
A process that uses genetic recombination to repair DNA breaks. A broken DNA end base-pairs with an intact homologous DNA sequence and uses the intact DNA as a template for repair synthesis, so restoring the integrity of the broken DNA end.

Hypermutation
Unusually high-frequency mutation. In the immune system, somatic hypermutation is part of a programmed mutagenesis strategy that increases antibody diversity and affinity. Hypermutation is a cell-type- and stage-specific process.

Mutator DNA polymerase
Any DNA polymerase with a high error rate, compared with the housekeeping DNA polymerases that carry out most of DNA replication.

Translesion DNA synthesis
Several kinds of damage to DNA block replication of the damaged strand by housekeeping DNA polymerases until the damage is repaired. Examples of such damage include abasic sites, alkylated bases and pyrimidine base dimers. If the DNA is not repaired, special DNA polymerases can allow synthesis across the damaged site ('translesion' synthesis).

the adaptive mutants; and recently discovered, a special inducible, mutator DNA polymerase (pol IV or DinB) that has homologues in all three domains of life[35] (archaea, bacteria and eukaryotes) (BOX 3). In this review, I describe — using the bacterial *lac* system as a model — some of the mechanisms that underlie adaptive genetic change and discuss how these mechanisms bear similarities to genetic instability in yeast and in some cancers and to somatic hypermutation in the immune system. They might also be important in bacterial evolution, especially the evolution of PHASE-VARIABLE PATHOGENS.

Adaptive point mutation at *lac*

When Lac$^-$ cells used in the *lac* frameshift reversion assay (described above and in BOX 2) are spread onto lactose-minimal medium, colonies of Lac$^+$ mutant cells appear during several days of incubation[12] (FIG. 1). The early colonies consist of growth-dependent Lac$^+$ mutants that are formed during liquid growth of the cultures before exposure to lactose medium, and the later ones consist of genuine adaptive mutants, as shown by the rigorous criteria outlined in BOX 2[13,36]. Most Lac$^+$ colonies carry point mutations[13,37,38] (FIG. 1) and the molecular mechanism that gives rise to them differs markedly from those that generate Lac$^+$ mutations in growing cells.

Recombination and DNA double-stranded breaks. Unlike growth-dependent Lac$^+$ reversion, adaptive point mutation in the *lac* system requires homologous recombination proteins of the *E. coli* RecBCD double-stranded-break-repair (DSBR) system[39–41]. Because the RecBCD enzyme complex loads onto DNA only at double-stranded ends (DSEs) (reviewed in REF. 42), this

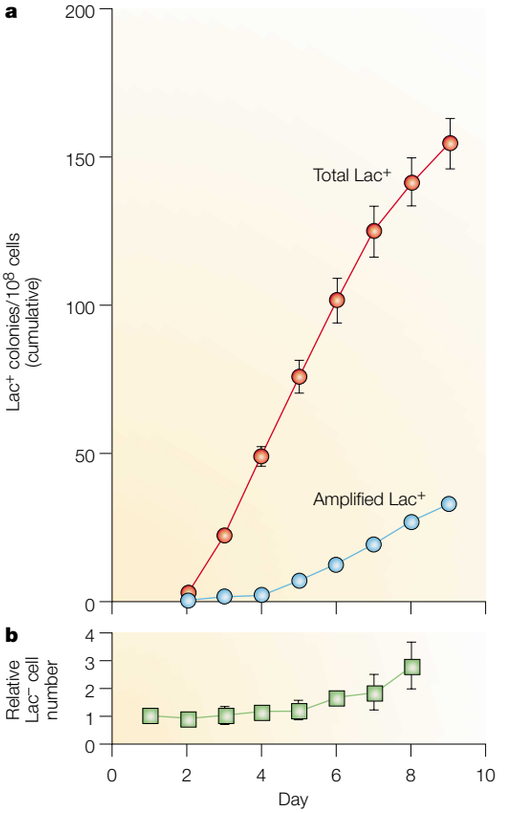


Figure 1 | **Adaptive point mutation and amplification in the *lac* frameshift system. a** | *Escherichia coli* cells that carry a *lac* +1 frameshift allele on an F′ EPISOME mutate to Lac$^+$ during starvation on lactose medium[12]. Growth-dependent Lac$^+$ mutants that occurred before exposure to the lactose plates form visible colonies by about two days. The colonies that emerge during the following days belong to two classes. Most of the early Lac$^+$ colonies (red circles) are adaptive point mutants. These occur by a recombination-dependent point mutation mechanism[39–41] and produce compensatory frameshift mutations[37,38]. On later days, an increasing fraction of the colonies are not point mutants but instead carry amplification to 20–50 direct repeats of a 7–40-kb region of DNA that contains the *lac* frameshift allele, which, owing to its leakiness, provides sufficient gene activity to allow growth on lactose medium[13,123] (blue circles, data from REF. 13). Both genetic changes are adaptive, as defined in BOX 2. **b** | This graph shows that the number of Lac$^-$ cells does not increase during the first five days of the experiment.

RecBCD-dependence implicates double-stranded breaks (DSBs) or DSEs as molecular intermediates[39]. Possible origins of DSEs during recombination-dependent adaptive mutation are discussed in BOX 4.

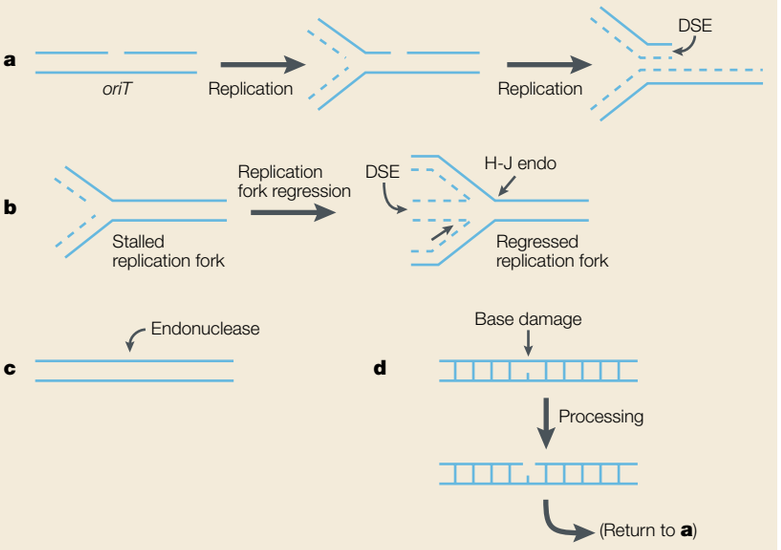
Replication and recombination. We suggested previously[39] that adaptive mutations might form by DNA polymerase errors that occur during replication that are initiated by recombinational strand invasion during DSBR (FIG. 2a,b). The idea that replication might begin through recombination, independently of standard replication origins, was speculative then, though not unprecedented. This had been well documented for phage T4

F′ CONJUGATIVE PLASMID
The F plasmid is a 100-kb extrachromosomal DNA replicon in bacteria that is transmissible between bacteria through conjugation (a specialized sexual process requiring cell–cell contact and mediated by conjugative plasmid-encoded proteins). F′ conjugative plasmids are F conjugative plasmids that have acquired, through recombination, a segment of DNA from the bacterial chromosome.

PHASE-VARIABLE PATHOGENS
Pathogenic microorganisms (both bacterial and eukaryotic) that evade the host immune system by frequent variation of their surface components (proteins, carbohydrates and lipids), called phase variation. The phase variations can be promoted by genetic changes, including mutation and recombination.

EPISOME
A replicon that can exist either extrachromosomally or when integrated into the bacterial chromosome.

Box 4 | **What is the origin of DNA DSBs in adaptive mutation?**



The double-stranded breaks (DSBs) or ends (DSEs) that promote adaptive mutation in the *lac* region could arise and promote mutation by any of many possible means. Replication into[47] (or other processing of [88]) a single-stranded (SS) nick at the origin of transfer (*oriT*) on the F′ plasmid is shown in a. (Dashed lines represent newly synthesized DNA.) A similar process at any SS-broken region on the chromosome could explain chromosomal hot spots for mutation. DSEs would be more common on the F′ (where *oriT* is nicked frequently) than on the chromosome, on which the SS breaks could arise from base damage (oxidative or other) and its repair, or by other means.

Stalled and REGRESSED REPLICATION FORKS[120,121] could provide RecBCD loading sites for adaptive mutation[39] (b), with or without further processing by cruciform-cleaving endonucleases — the Holliday junction resolvases (H-J endo)[48,122]. DSBs could be caused by enzymatic cleavage of DNA by any of many enzymes with double-stranded endonuclease activity, including the control of cell death (Ccd) enzyme, which is a GYRASE inhibitor that causes gyrase-mediated DSBs[90], restriction enzymes, or other enzymes not yet known (c). DSBs could also be caused by chemical damage (such as oxidative damage) with or without its repair by endonucleases (d).

(REF. 43), and had been proposed for phage λ (REF. 44) and *E. coli* (reviewed in REF. 45). Break-repair-induced replication is now recognized as a frequent route by which broken or stalled replication forks are repaired and restarted; in fact, break-repair-induced replication might represent the most frequent role for recombination in cells[46].

Models for association of DSBs and mutation

The model[39] in FIG. 2a,b, and many subsequent versions of it[40,41,47–49], has persisted as the dominant model for the origin of point mutations in the *lac* system. In this model, a DSB or DSE primes replication and generates a mutation *in cis*, directly associated with the DSE and the recombination event used to repair it. (How DSBs might form is discussed in BOX 4.) Such a direct association of DSBs and DSBR with the point mutations has not yet been shown in adaptive mutation in the *lac* system, although direct association of RecBCD-mediated DSBR with replication *in vivo* has been shown[50], and direct association of mutation with DSBR has been shown in yeast[51]. Because a direct association has not been shown in generating adaptive *lac* mutations, models that invoke the indirect association of DSBR, or its proteins, with adaptive mutation are also possible. An example of one is shown in FIG. 2c. In indirect models, RecBCD-dependent DSBR proteins are required for some stage of the process, but do not promote mutation directly *in cis* to their site of action. In the model in FIG. 2c, even recombination of DNA is not required.

Enzymatic milieu: MMR limitation and SOS

Simple repeat instability. The Lac$^+$ adaptive point mutations that revert a frameshift allele are nearly all –1 deletions in small mononucleotide repeats, whereas growth-dependent Lac$^+$ reversions are heterogeneous, including –1s, +2s, and larger insertions and deletions[37,38]. Mononucleotide-repeat instability is thought to reflect DNA polymerase errors formed by a TEMPLATE SLIPPAGE mechanism (reviewed by REF. 52), implying that the adaptive mutations are polymerase errors. Initially, the principal replicase of *E. coli*, pol III, was implicated[53,54], but recent work indicates that a special error-prone DNA polymerase (pol IV) is required (discussed below).

Transient mismatch repair limitation. Simple repeat instability is a hallmark of bacterial, yeast and human cells that lack post-replicative mismatch repair (MMR)[55,56]. MMR is the principal polymerase-error correction and mutation-prevention system of eubacteria and eukaryotes, and uses homologues of the *E. coli* MutL and MutS proteins in all these species[55,56]. The sequences of Lac$^+$ adaptive point mutations mimic growth-dependent Lac$^+$ reversions formed in MMR-defective mutants[57], but the adaptive mutants are not MMR defective[57–59]. This indicated a possible transient MMR deficiency[57], which was then shown to occur at the level of limiting MutL protein[60] (see REFS 61,62 for discussion of alternative interpretations, and REFS 63,64 for different environmental conditions under which MutS might become limiting for different kinds of MMR reactions).

REGRESSED REPLICATION FORK
A replication fork that, upon pausing (stalling) of replication, isomerizes such that the newly synthesized daughter strands base-pair with each other (not the templates on which they were synthesized).

DNA GYRASE
An enzyme that relieves supercoiling in DNA by creating a transient break in the double helix.

TEMPLATE SLIPPAGE
A model for the mechanism of frameshift mutation in which a DNA polymerase jumps to an incorrect next place on the template DNA during synthesis, thereby either adding or deleting bases in the nascent strand, the complements of which were present in the template.

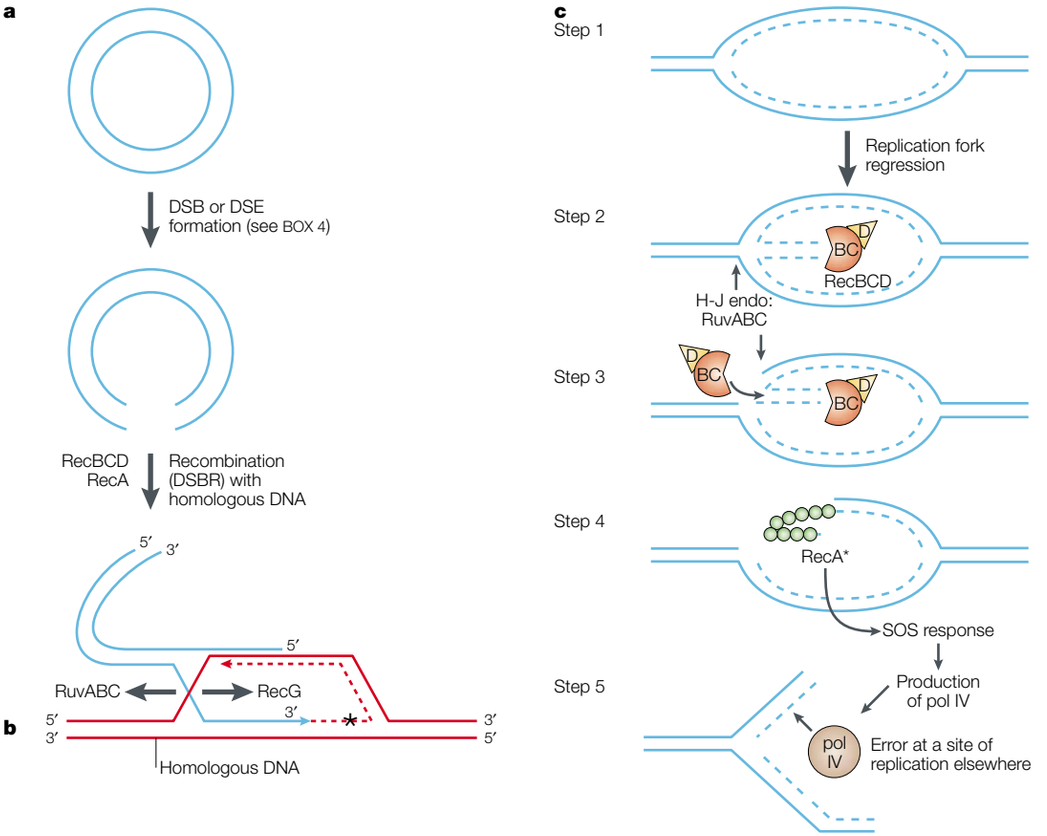


Figure 2 | **Two models for generating adaptive point mutations through recombination proteins.** Dashed lines represent newly synthesized DNA. **a, b** | Direct model: replication primed by recombination[39]. In **a**, the circular F′ (or chromosome) acquires a double-stranded break (DSB) or double-stranded end (DSE) by any of the possible ways outlined in BOX 4. DSBR, DSB repair. In **b**, RecBCD-mediated DSB-repair by homologous recombination allows strand invasion of a region of homologous DNA (perhaps present in a sister chromosome) and primes DNA replication, during which errors accumulate (asterisk). The error could be caused by the mutator DNA polymerase pol IV or by another DNA polymerase. Strand invasion requires RecBC, and RecA, which are required for recombination-dependent adaptive point mutation[39]. The branch migration activity of the RuvABC proteins is proposed to stabilize 3′-end invasion intermediates, which are postulated to prime replication; the opposite polarity branch migration activity of RecG is proposed to unwind and destroy the intermediates that lead to DNA replication[41]. These events might explain the requirement in adaptive point mutation for RuvABC and its depression by RecG[40,41]. DSBs and DSBR are directly associated with mutation in models such as this. In *Escherichia coli*, the homologous DNA used in recombination could be a sister molecule (present in about 30–40% of stationary-phase cells[124]), a duplicated region (present in 10^{-2}–10^{-3} of cells for any given DNA segment[125]), or even DNA that has been taken up from outside the cell (a process not known to occur in *E. coli* but that has not been ruled out). **c** | An indirect model: indirect association of recombination protein action and adaptive point mutation. Step 1, a replication fork stalls. Step 2, regression of the replication fork[120] provides a substrate for RecBCD[39,121]. Step 3, the regressed fork is cleaved by the Holliday-junction endonuclease (H-J endo) activity of RuvABC[39,122], which is required for adaptive point mutation[40,41]. Step 4, RecA loads onto RecBCD-exposed single strands, leading to activation of the SOS response, production of mutator pol IV and thus (step 5) to error-prone synthesis at some other replication site in DNA, not associated with any direct action of RecA, RecBCD or RuvABC. The SOS response is a DNA damage response in which about 42 DNA repair, recombination and mutation genes are upregulated in response to slowed replication or DNA damage[66].

Overproduction of MutL (with or without MutS) decreases adaptive mutation, but does not affect growth-dependent Lac$^+$ frameshift mutation, indicating that MutL becomes limiting for MMR function specifically during adaptive mutation[60,62]. Paradoxically, numbers of MutL molecules per cell do not decline in stationary phase when MutL becomes limiting (REF. 60 and references therein). The mechanism of transient MutL/MMR limitation during adaptive point mutation is an important unanswered question (see REFS 60,62,65), discussed further below.

The SOS response. SOS is the bacterial DNA-damage and cell-cycle checkpoint control response, induced in response to DNA damage or to the inhibition of DNA replication[66]. Induction of the SOS response de-represses at least 42 genes that function in DNA repair, recombination, mutation, translesion DNA synthesis and prevention of cell division — genes usually repressed by the transcriptional repressor LexA. Cells that are mutant for specific alleles of LexA or RecA, and are therefore unable to induce LexA-controlled genes (RecA stimulates the autoproteolysis of LexA), show reduced Lac$^+$ adaptive

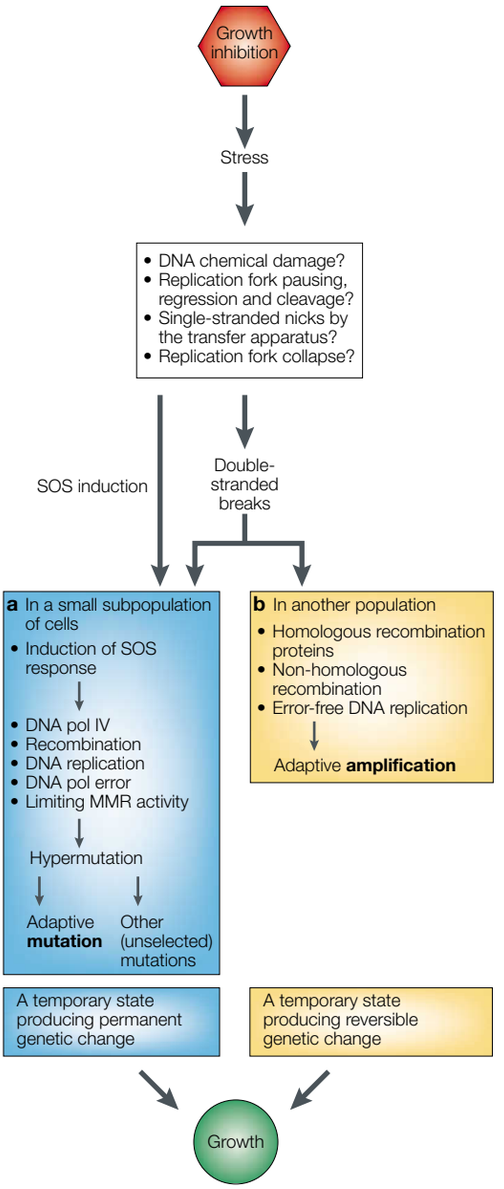


Figure 3 | **Models for adaptive point mutation and amplification.** Recombination-dependent adaptive point mutation (blue) and amplification (yellow) in the *lac* system of *Escherichia coli*. MMR, mismatch repair; pol, polymerase.

mutation[12,67]. RecA was thought to be the only protein controlled by LexA to be required at induced levels[12]. However, subsequent data showed that some other LexA controlled function(s) is required, indicating that a special mutation-promoting enzyme controlled by LexA might promote adaptive point mutations[67]. We shall see below that a LexA-regulated DNA polymerase that is expressed detectably only during an SOS response is required for Lac$^+$ adaptive mutation[65].

A special error-prone DNA polymerase

Two error-prone DNA polymerases, UmuD′C (pol V) and DinB (pol IV), and one high-fidelity DNA polymerase, pol II, are upregulated during the SOS response[66]. Pols IV and V belong to the new DinB/UmuDC superfamily of DNA polymerases that are found in eubacteria, archaea and eukaryotes. Pol V is a translesion polymerase (reviewed in REFS 35,66) (BOX 3) and is not required for adaptive Lac$^+$ reversion[12,67]. Pol IV is required specifically for adaptive point mutation, but not for growth-dependent Lac$^+$ reversion, other growth-dependent mutations, survival of ultraviolet or oxidative damage, or adaptive amplification (discussed below)[65]. Previous work that indicated a small effect of pol IV on growth-dependent mutation[68] used a POLAR ALLELE and might have measured adaptive mutations together with growth-dependent mutations (discussed in REF. 65). Pol IV is unable to carry out translesion synthesis *in vitro* but was suggested to function in translesion synthesis opposite a bulky DNA adduct[69]. Because that study[69] also used a *dinB* allele that inactivates other gene(s) downstream (see REF. 65), it remains to be determined whether *dinB* (encoding pol IV) was the gene responsible. So, it is not yet known whether pol IV has any role in DNA damage tolerance and translesion synthesis or whether it functions solely in mutation.

The adaptive point mutations due to pol IV resemble the frameshift component of the error spectrum of purified pol IV enzyme[65,70], indicating that pol IV might make the errors that become adaptive mutations (although pol IV also makes substitutions[70–72]). The idea of special, inducible mutation-promoting enzymes that accelerate evolution when needed[73,74] is supported by the discovery of this role for pol IV in adaptive mutation. Pol IV is the sole member of the DinB branch of the DinB/UmuDC superfamily for which a biological role (in adaptive mutation) has been assigned unambiguously. Pol IV has four mammalian homologues, three of which of unknown function (in this and two other subfamilies) — DINB1, RAD30b and REV1 — which are present in germline and lymphoid cells, and are considered below.

Does pol IV overwhelm MMR? The role of pol IV in adaptive mutation indicates one way that MMR could become limiting: errors made by pol IV might titrate MMR enzymes[65], as was seen with another mutator polymerase (one with inactivated proofreading activity)[75]. Evidence of MutL/MMR limitation was seen in one study in which pol IV was overexpressed[72], but not in another[71]. So, this and other possible mechanisms of MMR limitation[60,62] require further exploration.

Global hypermutation in a subpopulation

The data above do not address the controversial possibility that mutations might be directed preferentially to those genes that could favour survival in the selective environment[7]. This question was answered in the *lac* frameshift system by the discovery that mutations are not directed to[58,76], or even to the same DNA molecule as[58], the *lac* gene, which supports a random Darwinian process of mutation.

POLAR ALLELE
A mutant allele of a gene that decreases or eliminates the expression of additional gene(s) downstream. Polar alleles are common in prokaryotic operons (groups of genes transcribed in a single mRNA).

In a random, non-directed model for adaptive point mutation, only a small subpopulation of the stressed, starving cells is mutable (FIG. 3a). In this subpopulation, stress leading to DSBs (BOX 4 and FIG. 3) promotes SOS induction, which upregulates pol IV. DSBs are repaired by recombination, which promotes replication and polymerase errors that persist in a milieu of suboptimal MMR capacity. Mutations form near sites of DSBR, not directed particularly to the *lac* gene. This model predicts that mutations other than those at *lac* will occur, but would be rare in the main cell population and frequent in the mutable subpopulation that gives rise to adaptive Lac$^+$ mutants. This is similar to Hall's hypermutable state idea[15]. We[58], and then others[59,77], confirmed the model's central prediction that Lac$^+$ adaptive mutants — the proposed descendents of the hypermutable subpopulation — are peppered with unselected mutations throughout their genomes, whereas the Lac$^-$ cells that starved on the same plates are not. Frequencies of unselected ('secondary') mutations are about two orders of magnitude higher among Lac$^+$ mutants than in the main population of Lac$^-$ starved cells. These results mean, first, that stationary-phase mutations in this system are not directed to the *lac* gene — both adaptive and neutral mutations are formed. Second, some or all of the adaptive mutants arise in a subpopulation that is hypermutable relative to the main population (discussed further below). Third, because the bacterial chromosome is also mutated, mutations are not confined to the F′ plasmid that carries *lac* (discussed below).

The subpopulation. The subpopulation cells are transiently (not heritably) mutable, in that they produce adaptive mutants that are neither growth-dependent mutator mutants[58,59], nor mutators for adaptive mutation[78]. The hypermutable subpopulation is estimated to be between 10^{-3} and 10^{-4} of all cells[79,80]. This subpopulation differs from that in Hall's hypermutable state proposal in which cells either acquire an adaptive mutation or die[15]. In the *lac* system, death is not inevitable for cells that enter the mutable subpopulation but do not become Lac$^+$. Unselected stationary-phase mutants (that are still Lac$^-$) also accumulate during exposure to starvation on lactose-minimal medium, both on the F′ (REF. 76) and on the bacterial chromosome[81], by a recombination-dependent[76,81], and pol IV-dependent mechanism[81]. So, a similar mechanism forms Lac$^+$ adaptive mutations and also the unselected mutations on the F′ and on the chromosome.

The origin of the transiently hypermutable subpopulation is an important question. One attractive idea is that it is SOS induction that sets these cells apart and makes them mutable through pol IV (REFS 65,67). A starvation-induced SOS response (and mutability) have been described[33] and might be similar to that in the *lac* system.

How many subpopulations? Directed mutations? The idea that mutations are directed to, or near to, genes that could confer an advantage if mutated (directed mutation hypothesis, BOX 1) almost returned last year with the proposal that most Lac$^+$ mutants do not arise in the hypermutable subpopulation[59,82]. If this were true, then the only remaining evidence that mutations are not directed to *lac* would be from a study that shows mutation occurring in a non-*lac* gene on the F′ (REF. 76). However, mutations in an unselected gene linked to *lac* on the F′ seem not to be independent of *lac* mutation[79]. Therefore, those unselected mutations on the F′ might occur only during events that also produce adaptive mutations next to them. This would be a kind of (loosely) gene-directed mutation. The idea that there are two mutable populations, and that mutations might occur only on the F′, are contradicted by two findings in a recent report[81]. First, a chromosomal unselected gene (unlinked to *lac*) is also mutated during lactose selection in a process that requires recombination proteins and pol IV. Therefore, recombination-dependent stationary-phase mutation is not confined to *lac* and the surrounding region — it also occurs on the chromosome. Second, the idea that there are two mutable populations (a minor hypermutable one that generates selected and other mutations, and a major one that produces selected mutations with few unselected mutations)[59,82] was proposed to explain a greater than expected number of triple mutants (Lac$^+$ mutants carrying two secondary mutations) relative to double mutants (Lac$^+$ mutants carrying one secondary mutation) and single Lac$^+$ mutants. This conclusion was based on very few data (only four triple mutants in one study[59] and five in another[58]), so more data would be required to distinguish between the one- and two-mutable population models[79,80]. However, even if additional data support the apparent excess of triple mutants, this could be interpreted in terms of a one-subpopulation model in which cells spend varying lengths of time in the hypermutable state[81]. Indeed, there are data that support this 'sliding scale' model, in which more mutations in a cell are caused by longer periods spent in the hypermutable condition[81] (and see REF. 77).

Hot and cold spots in bacterial chromosomes

Genome-wide hypermutation in Lac$^+$ adaptive mutants[58,59,77] occurs with markedly uneven frequency per unit length of DNA. A loss-of-function mutation in the uracil phosphoribosyltransferase (*upp*) gene is about ten times more frequent than a loss-of-function mutation in the entire maltose (Mal) or xylose (Xyl) fermentation regulons (more than seven genes for Mal$^-$ and fewer for Xyl$^-$)[58]. This indicates that some sites or areas are 'hot', whereas others are 'cold' for hypermutation. Hot and cold sites in the chromosome might be caused by proximity to sites at which DSBs occur[83] (FIG. 4). This could explain why the *lac* operon (in F$^-$ cells) does not show recombination-dependent mutation in stationary phase[59,84,85], whereas the *upp* site (in F′-carrying cells) does[81]. (Alternatively, the presence of an F′ in one[81], and not the other[84,85], experiment might be important[77].) If DSBs provoke stationary-phase mutations locally, then there is some degree of targeting — by DSBs — of stationary-phase mutation in this system. Evidence that DSBs direct mutations now exists in somatic hypermutation in immunoglobulin genes and in yeast.

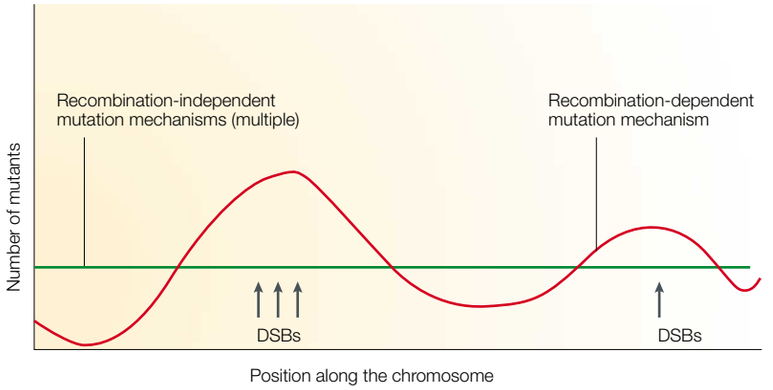


Figure 4 | **Hypothesis: hot and cold spots in the *Escherichia coli* chromosome are dictated by proximity to double-stranded breaks.** Chromosomal hot spots for recombination-protein-dependent mutation in stationary phase (red line) might fall in regions that are accessible to double-stranded breakage (DSB) in stationary phase (arrows), whereas cold spots (sites at which stationary-phase mutation is independent of Rec proteins, green line) might lie in regions broken less frequently. Accessibility to breaks could be determined by NUCLEOID structure (exposure of DNA to damaging agents or proteins), proximity to specific sequences that interact with enzymes that result in breakage (for example, replication pause sites or promoters), or any of many other possibilities that could be non-uniformly distributed.

Sex and the mutable bacterium

Adaptive mutation in the *lac* frameshift system requires transfer (Tra) proteins[84,86], but not CONJUGATIVE TRANSFER of the F′-carrying *lac* [84,85,87,88]. This, plus the apparent lack of recombination-dependent adaptive mutation at two chromosomal sites[84,86], led to the speculation that only the F′ (and not chromosomal sites) would be affected by recombination-dependent stationary-phase mutation[59,85,86]. This is contradicted by the discovery of recombination- and pol IV-dependent stationary-phase mutation in the *E. coli* chromosome[81]. Furthermore, chromosomal mutations form independently of Lac$^+$ mutations on the F′ (REF. 79) and are not correlated with Hfr formation (integration of the F′ into the bacterial chromosome), indicating that the chromosome and the F′ are not even connected during chromosomal mutation[89]. So, recombination-dependent point mutation is not confined to conjugative plasmids and promotes genetic change in the chromosomal genome.

The Tra proteins probably function in promoting DNA breaks that become DSBs (BOX 4), which could promote mutations directly[39,40,47,48,83,88] (FIG. 2a,b), or indirectly by inducing the SOS response required for pol IV upregulation[67] (FIG. 2c). Rec-dependent mutation occurs at higher frequency on the F′ (REFS 12,76) than the chromosome[81], in support of a direct, *in cis* mutation stimulation by the Tra proteins. Additionally, *trans*-acting F-encoded functions might also promote adaptive mutation[67,77]. Candidates include the DSB-producing TOPOISOMERASE inhibitor protein — Ccd (REF. 90), an F-encoded single-stranded DNA-binding protein[91] and other DNA metabolism proteins[67,91]. This could be generally important both in bacterial evolution and non-bacterial genomes, because the latter carry homologues of genes encoded by bacterial plasmids[66].

Adaptive amplification

A second, fundamentally different mode of adaptive genetic change — adaptive gene amplification — was shown recently in *E. coli*. Adaptive gene amplification occurs in the *lac* frameshift system[13] (FIG. 1). The leaky mutant *lac* allele can be amplified to 20–50 copies of 7–40-kb direct DNA repeats, providing sufficient gene activity for growth on lactose medium without acquisition of a Lac$^+$ point mutation. This process is adaptive (as defined in BOX 1), and is the first system in which gene amplifications have been shown by stringent criteria (outlined in BOX 2) to be induced, rather than simply selected, in response to a selective environment (although the possibility had long been postulated[74,92,93] and reviewed by REFS 13,94). The idea that amplification is a precursor to adaptive point mutation, as was proposed in one model[49], is now contradicted by several lines of evidence[13]. One of three tests[13] that the model failed is that cells carrying amplification do not readily produce adaptive point mutants under selective conditions, as would be expected if amplified DNA were an intermediate in the point mutation process. Amplification is a more flexible genomic alteration than point mutation; it can be returned to single copy by recombination and can allow evolution of a gene copy while an intact copy of the gene is retained[95]. Adaptive amplification is likely to be an important mode of adaptive genetic change in evolution and development.

A few points are already apparent regarding the mechanism of adaptive amplification of *lac*. First, it is unlike adaptive point mutation in that cells carrying the amplification are not hypermutated in unselected genes[13], and in that neither the SOS response nor pol IV is required[65]. Second, the junction fragments of the repeat units are mapped to regions that must include non-homologous joints[13], as seen previously[92]. However, dependence on homologous recombination functions is implied (although not yet shown rigorously) by previous findings that total adaptive Lac$^+$ colonies do not appear in the absence of RecA, RecBCD enzyme, and RuvAB and C recombination proteins[39–41]. This (possible) RecBC dependence implies that DSBs are an intermediate. Perhaps the homologous recombination proteins process DSBs in a non-homologous recombination event that begins amplification (FIG. 3b). One could imagine that adaptive point mutations might occur when a homologous sequence is present for the DSB to recombine with (for example, a sister chromosome), and that amplification might result when it is not. Unlike mammals, *E. coli* recombination shows a strong preference for homologous over non-homologous recombination (for example, REF. 96).

Parallels with amplification in mammals?

Amplification is an important manifestation of chromosomal instability prevalent in many human cancers[97] and some of the features of adaptive Lac amplification parallel mammalian DNA amplification. DSBs are implied as an intermediate (above) and have been shown to promote mammalian DNA amplification[94]. Induction of mammalian amplification by selective agents is corre-

NUCLEOID
Region in prokaryotes in which the DNA is concentrated. Unlike a nucleus, it is not bound by a membrane.

CONJUGATIVE TRANSFER
A specific process by which proteins encoded by the conjugative plasmid DNA (transfer or Tra proteins) allow passage of conjugative plasmid DNA, and any DNA contiguous with it, into another bacterium.

TOPOISOMERASES
Enzymes of two types that can remove (or create) supercoiling in duplex DNA by creating transitory breaks in one (type I) or both (type II) strands of the sugar-phosphate backbone.

BACTERIOSTATIC
Capable of inhibiting bacterial growth (but not necessarily capable of killing bacteria).

lated with the ability of those agents to produce chromosomal breaks[98]. Understanding the mechanism in the more tractable bacterial system might be highly informative for understanding this important manifestation of chromosomal instability in mammals.

Bacterial evolution and pathogenicity

The adaptive point mutation mechanism at *lac* might pertain to microbial evolution, particularly of pathogenic bacteria. Many phase-variable pathogens have simple repeated sequences that flank genes that they regulate by frameshift mutation[99,100]. These 'contingency genes' (used under stress) provide phase variations that allow evasion of the immune system. These bacteria might use adaptive mutation strategies similar to the point mutations discussed above. Two of them, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, have one or more genes homologous to *dinB* (discussed in REF. 65 and references therein). For many pathogenic bacteria, antibiotic resistance is also achieved by point mutation mechanisms[101] and could be induced adaptively. Even antibiotics that cause lethality (which should prevent adaptive mutation mechanisms) can be merely BACTERIOSTATIC at lower concentrations, such that stress-promoted mutation mechanisms might be significant in the development of resistance in clinical environments[101]. Adaptive mutations also resemble the phenomenon of mutations that confer a growth advantage in stationary phase (GASP)[102]. These are generally substitution mutations. However, frameshifts are probably not the sole contribution of recombination-dependent adaptive mutation[65], because pol IV causes substitutions as well as frameshifts[70–72].

How important are environmentally inducible mutation mechanisms to bacterial evolution, compared to the selection of pre-existing mutator mutants? Although mutator mutants are found among wild bacteria, they represent the minority (for example, REFS 103,104 and references therein). Most mutants (80–99%) are not mutators, so transient, inducible (adaptive) mutation strategies might contribute substantially[13,78].

Mutations directed by transcription

Amino-acid starvation elicits the 'stringent' response in bacteria, which includes the transcriptional upregulation of many genes, including those for amino-acid biosynthesis[105]. Some genes transcribed during the stringent response are hypermutated relative to non-transcribed genes, in a process that requires their transcription[25]. Artificially induced transcription also induces mutation in this system[25] and in yeast[106], the latter requiring the error-prone DNA polymerase REV3. Transcription-promoted mutation is a way in which mutations could be directed (loosely) to genes, the products of which are needed, and so could be part of a gene-directed, adaptive mutation strategy[107]. Although both beneficial and detrimental mutations would increase in those genes, deleterious mutations in other genes that might be required after the stress is surmounted, and that could not confer adaptive phenotypes if mutated, would be reduced. In both the yeast and bacterial systems, the mechanisms of transcription-associated mutation are unlike recombination-dependent adaptive point mutation in their independence of recombination proteins, but the yeast system resembles Lac point mutation in its requirement for an error-prone DNA polymerase, REV3. Interestingly, a more specifically targeted mutation mechanism is seen in somatic hypermutation (below). In that case, promoters and transcription might be important, by virtue of helping to promote DSBs that target mutation in a mechanism apparently similar to that in the *lac* adaptive mutation system.

Adaptive mutation in yeast

Adaptive mutation has been described in a few yeast assay systems[26–32] and a small amount of mechanistic information has been reported. In one system, the replicative DNA pol δ has been implicated in synthesis during adaptive mutation[29]. (Yeast polymerases of the DinB/UmuDC superfamily have not been tested.) In another system, the parallel to recombination-dependent mutation at *lac* seems to be striking: adaptive reversions of a net +1 frameshift mutation in a lysine biosynthesis gene are mostly –1 deletions in mononucleotide repeats, reportedly similar to those of MMR-defective yeast, whereas growth-dependent reversions are more heterogeneous[32]. However, another group studying the same allele sees similar reversion spectra in adaptive and growth-dependent mutants[27], necessitating a better understanding of possible differences between these studies. Also in yeast, mutations promoted by DSBR are well documented and require the *REV3* gene that encodes a subunit of DNA pol ζ (REF. 51), an error-prone DNA polymerase[35].

Box 5 | **Outstanding questions**

- **How many mechanisms of adaptive genetic change are there?**
- **What is the mechanism of adaptive amplification in *Escherichia coli*, and is it similar in mammals and during cancer?**
- **Is recombination-dependent adaptive point mutation conserved between prokaryotes and eukaryotes?**
- **How similar is recombination-dependent adaptive mutation to somatic hypermutation in the immune system?**
- **Do phase-variable pathogenic bacteria use adaptive frameshift mutation? Do they use DinB/UmuDC homologues?**

In recombination-dependent adaptive point mutation:

- **What is the origin of double-stranded breaks (DSBs)?**
- **Are DSBs directly associated with mutation?**
- **How does mismatch repair become limiting?**
- **Does this also occur in the yeast system?**
- **How is the hypermutable subpopulation differentiated?**
- **Do most or few adaptive mutants arise from the hypermutable subpopulation?**
- **How is the environment sensed and the signal transduced to effect genetic change?**
- **What triggers the SOS response?**
- **Is inducible evolution the main function of *E. coli* pol IV (DinB)?**
- **What are the functions of the mammalian DinB/pol IV homologues?**

Multicellular eukaryotes

Adaptive mutation and cancer. The parallels between adaptive mutation and cancer have been noted[37,108–110], the key parallel being that acquisition of mutations in a growth-limited state allows cells to proliferate. The precedent for MMR modulation in *E. coli* raises an important question about the possibility that environmental perturbation of MMR occurs in human cancers. Cancers that show simple repeat instability are promoted by heritable MMR defects, but not all such cancers are associated with MMR gene mutations (reviewed in REFS 55,56). Some of these might result from environmentally induced depression of MMR.

Human DinB homologues. Humans have three *E. coli* pol IV homologues of unknown function, in the DinB/UmuDC/Rad30/Rev1 superfamily of DNA polymerases[35], as well as a homologue known to carry out translesion synthesis (the tumour suppressor protein XP-V). DinB1 or pol κ, a true DinB orthologue, is found in germline and lymphoid cells. Could the germline pol IV orthologue promote germ-cell transmissible mutations? Drake and others have argued that mutation rates are regulated[111]. Although adaptive mutation seems counter-intuitive for multicellular organisms, which have a separate germ line, it is not impossible. Selection on gametes is extremely stringent (demanding successful development); the deleterious mutations expected by an inducible mutation process could therefore be culled. Because somatic mutation is disadvantageous, contributing to ageing and cancer, it is conceivable that the optimal level of mutation in germ cells is higher than in somatic tissue (but see REF. 112). Such differential mutability could be achieved by germline expression of a mutator polymerase.

An alternative possible role for DinB1 in the mammalian germ line is indicated by the recent discovery of apparent hypermutation in gamete protein genes that control fertilization of eggs by sperm. These highly variable proteins form a lock and key system by which eggs apparently exclude most sperm, and some sperm succeed in fertilization[113]. Something like an evolutionary arms race might occur between the male and female genes[113], rather like that between the immune system and its targets. DinB1 might, therefore, be part of a programmed mutation mechanism used in this battle between the sexes.

Somatic hypermutation. Programmed mutation is a conspicuous feature of the generation of antibody diversity, with parallels to adaptive mutation in the *E. coli lac* system[114]. The postulate that somatic hypermutation is caused by recombinational repair of breaks (reviewed in REF. 114) has garnered strong support from recent reports of DNA breaks as intermediates in the process (reviewed in REF. 115). These might be associated with transcription, a postulate not tested in the *lac* system. The possibility that human DinB homologues make polymerase errors that become the mutations is focusing on the Rad30-subfamily member pol ι (REF. 116) and others.

Phenotypic suppression mechanisms. In *Drosophila* and yeast, highly conserved protein-folding factors influence whether mutations have phenotypic consequences, by influencing whether a mutant protein folds normally (for example, REF. 117 and references therein). If, as in *E. coli*, most adaptive mutation mechanisms generate many unselected mutations, in addition to any adaptive ones, these protein-folding and quality control systems might be crucial to surviving bouts of adaptive mutation by reshaping mutant proteins into functional forms. Also, their regulation might allow previous episodes of mutability to be expressed phenotypically when advantageous[117]. It seems reasonable to expect an interplay between these systems and inducible mutation systems.

Future directions

In the best-studied systems of adaptive genetic change in *E. coli*, we have seen a few mechanisms of environmentally inducible mutability, each of them working differently, and causing unselected as well as adaptive mutations. So, generally, adaptive and stationary-phase mutations seem to be a general strategy of mutagenic stress responses, not a specific (single) mechanism or response. The parallels between different systems are becoming clearer and prompt scores of new questions, a small sample of which is shown in BOX 5. Evolutionary principles have not, apparently, been violated by any of these phenomena of adaptive mutation. Although not exactly like the Haggunenons, all organisms' genomes might be much more flexible and responsive than previously suspected.

Update — added in proof

Three recent papers provide diverse evidence for the possible involvement of two of the four DinB/UmuDC superfamily polymerases that are present in vertebrates, and the REV3-encoded pol ζ (REF. 126), the RAD30A-encoded pol ε (or XPV, the polymerase that is defective in individuals with the xeroderma pigmentosum, variant form, precancerous syndrome)[127,128], and the *E. coli* DinB orthologue, DINB1 or pol κ (REF. 127). The paradigm of mutations promoted by special error-prone polymerases during DNA break repair, originally proposed for stationary-phase mutation in the *lac* system[39], and the identifications of DinB/UmuDC polymerase superfamily members in this process in microbial systems[51,65], is now garnering substantial support and generalization in the mechanism of somatic hypermutation[126–128]. An additional paper that implicates DinB in mutation in the *lac* system has been cited[129], but is not yet available as this review goes to press.

Links

FURTHER INFORMATION Fluctuation test of Luria and Delbrück

ENCYCLOPEDIA OF LIFE SCIENCES Homologous genetic recombination during bacterial conjugation | Recombinational DNA repair in bacteria: postreplication | Eukaryotic recombination: initiation by double-strand breaks | SOS response | Topoisomerases

1. Adams, D. *The Hitch-hiker's Guide to the Galaxy: The Original Radio Scripts* (Pan Books, Ltd, London, 1985).
2. Mayr, E. *The Growth of Biological Thought, Diversity, Evolution, and Inheritance* (Belknap, Cambridge, Massachusetts, 1982).
3. Luria, S. E. & Delbrück, M. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28**, 491–511 (1943).
4. Newcombe, H. B. Origin of bacterial variants. *Nature* **164**, 150–151 (1949).
5. Lederberg, J. & Lederberg, E. M. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63**, 399–406 (1952).
References 3–5 are classic papers that provided the first evidence that mutations can occur independently of specific interaction with an environment in which the mutation is selected. They did not look for mutations induced by contact with a selective environment.
6. Shapiro, J. A. Observations on the formation of clones containing araB–lacZ fusions. *Mol. Gen. Genet.* **194**, 79–90 (1984).
7. Cairns, J., Overbaugh, J. & Miller, S. The origin of mutants. *Nature* **335**, 142–145 (1988).
References 6 and 7 inaugurated the modern study of adaptive mutation in bacteria. However, reference 11 showed that most putative adaptive mutations in reference 7 were really growth dependent.
8. Novick, A. & Szilard, L. Experiments on spontaneous and chemically induced mutations of bacteria growing in the chemostat. *Cold Spring Harbor Symp. Quant. Biol.* **16**, 337–343 (1951).
9. Ryan, F. J. Distribution of numbers of mutant bacteria in replicate cultures. *Nature* **169**, 882–883 (1952).
10. Ryan, F. Spontaneous mutations in non-dividing bacteria. *Genetics* **40**, 726–738 (1955).
11. Prival, M. J. & Cebula, T. A. Adaptive mutation and slow-growing revertants of an *Escherichia coli lacZ* amber mutant. *Genetics* **144**, 1337–1341 (1996).
12. Cairns, J. & Foster, P. L. Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics* **128**, 695–701 (1991).
Description of the *lac* frameshift reversion assay for adaptive mutation and demonstration of a role for the SOS response, also explored in reference 67.
13. Hastings, P. J., Bull, H. J., Klump, J. R. & Rosenberg, S. M. Adaptive amplification: an inducible chromosomal instability mechanism. *Cell* **103**, 723–731 (2000).
Shows that amplification can be an adaptive response. It also disproves predictions of a model (reference 49) in which amplification is postulated to lead to late Lac reversions through a growth-dependent mutation process.
14. Hall, B. G. Adaptive evolution that requires multiple spontaneous mutations. I. Mutations involving an insertion sequence. *Genetics* **120**, 887–897 (1988).
15. Hall, B. G. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* **126**, 5–16 (1990).
16. Boe, L. & Marinus, M. G. Role of plasmid multimers in mutation to tetracycline resistance. *Mol. Microbiol.* **5**, 2541–2545 (1991).
17. Prival, M. J. & Cebula, T. A. Sequence analysis of mutations arising during prolonged starvation of *Salmonella typhimurium*. *Genetics* **132**, 303–310 (1992).
18. Jayaraman, R. Cairnsian mutagenesis in *Escherichia coli*: genetic evidence for two pathways regulated by *mutS* and *mutL* genes. *J. Genet.* **71**, 23–41 (1992).
19. Bridges, B. Spontaneous mutation in stationary-phase *Escherichia coli* WP2 carrying various DNA repair alleles. *Mutat. Res.* **302**, 173–176 (1993).
20. Maenhaut-Michel, G., Blake, C. E., Leach, D. R. & Shapiro, J. A. Different structures of selected and unselected araB–lacZ fusions. *Mol. Microbiol.* **23**, 133–145 (1997).
21. Galitski, T. & Roth, J. R. A search for a general phenomenon of adaptive mutability. *Genetics* **143**, 645–659 (1996).
22. Kasak, L., Horak, R. & Kivisaar, M. Promoter-creating mutations in *Pseudomonas putida*: a model system for the study of mutation in starving bacteria. *Proc. Natl Acad. Sci. USA* **94**, 3134–3139 (1997).
23. Hall, B. G. Adaptive mutagenesis at ebgR is regulated by PhoPQ. *J. Bacteriol.* **180**, 2862–2865 (1998).
24. Massey, R. C., Rainey, P. B., Sheehan, B. J., Keane, O. M. & Dorman, C. J. Environmentally constrained mutation and adaptive evolution in *Salmonella*. *Curr. Biol.* **9**, 1477–1480 (1999).
References 14–24 report stationary-phase mutations, many of which do not form by mechanisms similar to the *lac* system. Included are several that have not been distinguished mechanistically from growth-dependent mutation. Reference 25 describes transcription-associated mutation in bacteria.
25. Wright, B. E., Longacre, A. & Reimers, J. M. Hypermutation in derepressed operons of *Escherichia coli* K12. *Proc. Natl Acad. Sci. USA* **96**, 5089–5094 (1999).
26. Steele, D. F. & Jinks-Robertson, S. An examination of adaptive reversion in *Saccharomyces cerevisiae*. *Genetics* **132**, 9–21 (1992).
27. Greene, C. N. & Jinks-Robertson, S. Comparison of spontaneous and adaptive mutation spectra in yeast. *J. Genet.* **78**, 51–55 (1999).
28. Hall, B. G. Selection-induced mutations occur in yeast. *Proc. Natl Acad. Sci. USA* **89**, 4300–4303 (1992).
29. Baranowska, H., Policinska, Z. & Jachymczyk, W. J. Effects of the *CDC2* gene on adaptive mutation in the yeast *Saccharomyces cerevisiae*. *Curr. Genet.* **28**, 521–525 (1995).
30. Heidenreich, E. & Wintersberger, U. Starvation for a specific amino acid induces high frequencies of rho^- mutants in *Saccharomyces cerevisiae*. *Curr. Genet.* **31**, 408–413 (1997).
31. Heidenreich, E. & Wintersberger, U. Replication-dependent and selection-induced mutations in respiration-competent and respiration-deficient strains of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **260**, 395–400 (1998).
32. Heidenreich, E. & Wintersberger, U. Adaptive reversion of a frameshift mutation in arrested *Saccharomyces cerevisiae* cells by simple deletions in mononucleotide repeats. *Mutat. Res.* **473**, 101–107 (2001).
The seven papers above report on adaptive mutations in yeast.
33. Taddei, F., Halliday, J. A., Matic, I. & Radman, M. Genetic analysis of mutagenesis in aging *Escherichia coli* colonies. *Mol. Gen. Genet.* **256**, 277–281 (1997).
In this paper, RecA- and RecB-dependent stationary-phase mutations are described that might be similar to recombination-dependent stationary-phase point mutation in the *lac* system. These occur in cells with or without a conjugative plasmid. There are some differences in genetic requirements between these and the *lac* system mutations.
34. Lamrani, S. *et al.* Starvation-induced Mucts62-mediated coding sequence fusion: a role for ClpXP, Lon, RpoS and Crp. *Mol. Microbiol.* **32**, 327–343 (1999).
35. Friedberg, E. C., Feaver, W. J. & Gerlach, V. L. The many faces of DNA polymerases: strategies for mutagenesis and for mutational avoidance. *Proc. Natl Acad. Sci. USA* **97**, 5681–5683 (2000).
36. McKenzie, G. J., Lombardo, M.-J. & Rosenberg, S. M. Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. *Genetics* **149**, 1563–1565 (1998).
37. Rosenberg, S. M., Longerich, S., Gee, P. & Harris, R. S. Adaptive mutation by deletions in small mononucleotide repeats. *Science* **265**, 405–407 (1994).
38. Foster, P. L. & Trimarchi, J. M. Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. *Science* **265**, 407–409 (1994).
39. Harris, R. S., Longerich, S. & Rosenberg, S. M. Recombination in adaptive mutation. *Science* **264**, 258–260 (1994).
40. Foster, P. L., Trimarchi, J. M. & Maurer, R. A. Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. *Genetics* **142**, 25–37 (1996).
41. Harris, R. S., Ross, K. J. & Rosenberg, S. M. Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. *Genetics* **142**, 681–691 (1996).
References 37 and 38 show that the sequences of Lac adaptive mutations differ from growth-dependent Lac reversions. The former show simple-repeat instability, which is characteristic of template slippage polymerase errors. References 39–41 show the specific requirement for recombination and double-stranded break-repair proteins for stationary-phase Lac reversion.
42. Kowalczykowski, S. C. Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem. Sci.* **25**, 156–165 (2000).
43. Mosig, G. The essential role of recombination in phage T4 growth. *Annu. Rev. Genet.* **21**, 347–371 (1987).
44. Skalka, A. in *Mechanisms in Recombination* (ed. Grell, R. R.) 421–432 (Plenum, New York, 1974).
45. Kogoma, T. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiol. Mol. Biol. Rev.* **61**, 212–238 (1997).
46. Radding, C. M. (ed.) *National Academy of Sciences Colloquium: Links Between Recombination and Replication* (Proc. Natl Acad. Sci. USA, 2001, in the press).
47. Kuzminov, A. Collapse and repair of replication forks in *Escherichia coli*. *Mol. Microbiol.* **16**, 373–384 (1995).
48. Rosenberg, S. M., Harris, R. S., Longerich, S. & Galloway, A. M. Recombination-dependent mutation in non-dividing cells. *Mutat. Res.* **350**, 69–76 (1996).
49. Andersson, D. I., Slechta, E. S. & Roth, J. R. Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon. *Science* **282**, 1133–1135 (1998).
50. Motamedi, M., Szigety, S. K. & Rosenberg, S. M. Double-strand-break repair in *Escherichia coli*: physical evidence for a DNA replication mechanism *in vivo*. *Genes Dev.* **13**, 2889–2903 (1999).
51. Holbeck, S. L. & Strathern, J. N. A role for REV3 in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. *Genetics* **147**, 1017–1024 (1997).
DSB-repair-promoted mutation in yeast, requiring DinB/UmuDC/Rad30/Rev1 DNA polymerase superfamily member, Rev3.
52. Ripley, L. S. Frameshift mutation: determinants of specificity. *Annu. Rev. Genet.* **24**, 189–213 (1990).
53. Foster, P. L., Gudmundsson, G., Trimarchi, J. M., Cai, H. & Goodman, M. F. Proofreading-defective DNA polymerase II increases adaptive mutation in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **92**, 7951–7955 (1995).
54. Harris, R. S., Bull, H. J. & Rosenberg, S. M. A direct role for DNA polymerase III in adaptive reversion of a frameshift mutation in *Escherichia coli*. *Mutat. Res.* **375**, 19–24 (1997).
55. Modrich, P. & Lahue, R. Mismatch repair in replication fidelity, genetic recombination, and cancer. *Annu. Rev. Biochem.* **65**, 101–133 (1996).
56. Fishel, R. Signaling mismatch repair in cancer. *Nature Med.* **11**, 1239–1241 (1999).
57. Longerich, S., Galloway, A. M., Harris, R. S., Wong, C. & Rosenberg, S. M. Adaptive mutation sequences reproduced by mismatch repair deficiency. *Proc. Natl Acad. Sci. USA* **92**, 12017–12020 (1995).
58. Torkelson, J. *et al.* Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* **16**, 3303–3311 (1997).
59. Rosche, W. A. & Foster, P. L. The role of transient hypermutators in adaptive mutation in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **96**, 6862–6867 (1999).
References 58 and 59, and also reference 77, provide evidence that Lac^+ adaptive mutants of *Escherichia coli* carry high frequencies of unselected mutations, which are not carried by starved cells that remained Lac^-. This indicates that some or all Lac^+ adaptive mutants originate in a globally hypermutable cell subpopulation. The data of reference 58 show hot and cold sites for this hypermutation.
60. Harris, R. S. *et al.* Mismatch repair protein MutL becomes limiting during stationary-phase mutation. *Genes Dev.* **11**, 2426–2437 (1997).
Evidence that cells undergoing adaptive mutation in the *lac* system have transient mismatch repair deficiency that can be alleviated by overproduction of MutL protein. See also reference 57. The interpretation of the data is criticized in reference 61, and this criticism is rebutted in reference 62.
61. Foster, P. L. Are adaptive mutations due to a decline in mismatch repair? The evidence is lacking. *Mutat. Res.* **436**, 179–184 (1999).
62. Harris, R. S. *et al.* Mismatch repair is diminished during stationary-phase mutation. *Mutat. Res.* **437**, 51–60 (1999).
63. Zhao, J. & Winkler, M. E. Reduction of GC→TA transversion mutation by overexpression of MutS in *Escherichia coli* K-12. *J. Bacteriol.* **182**, 5025–5028 (2000).
64. Brègeon, D., Matic, I., Radman, M. & Taddei, F. Mismatch repair: genetic defects and down regulation. *J. Genet.* **78**, 21–28 (1999).
65. McKenzie, G. J., Lee, P. L., Lombardo, M.-J., Hastings, P. J. & Rosenberg, S. M. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol. Cell* **7**, 571–579 (2001).
Shows that pol IV or DinB, of the DinB/UmuDC DNA polymerase superfamily, is required specifically for

most stationary-phase mutation at *lac*.
66. Sutton, M. D., Smith, B. T., Godoy, V. G. & Walker, G. C. The SOS response: recent insights into UmuDC-dependent mutagenesis and DNA damage tolerance. *Annu. Rev. Genet.* **34**, 479–497 (2000).
67. McKenzie, G. J., Harris, R. S., Lee, P. L. & Rosenberg, S. M. The SOS response regulates adaptive mutation. *Proc. Natl Acad. Sci. USA* **97**, 6646–6651 (2000).
68. Strauss, B. S., Roberts, R., Francis, L. & Pouryazdanparast, P. Role of the dinB gene product in spontaneous mutation in *Escherichia coli* with an impaired replicative polymerase. *J. Bacteriol.* **182**, 6742–6750 (2000).
69. Napolitano, R., Janel-Bintz, R., Wagner, J. & Fuchs, R. P. P. All three SOS-inducible DNA polymerases (pol II, pol IV, and pol V) are involved in induced mutagenesis. *EMBO J.* **19**, 6259–6265 (2000).
70. Wagner, J. *et al.* The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis. *Mol. Cell* **4**, 281–286 (1999).
71. Kim, S.-Y. *et al.* Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an SOS gene product (DinB/P) enhances frameshift mutations in the absence of any exogenous agents that damage DNA. *Proc. Natl Acad. Sci. USA* **94**, 13792–13797 (1997).
72. Wagner, J. & Nohmi, T. *Escherichia coli* DNA polymerase IV mutator activity: genetic requirements and mutational specificity. *J. Bacteriol.* **182**, 4587–4595 (2000).
Reference 70 shows that DinB is an error-prone polymerase. References 71 and 72 show that DinB protein causes mutations simply by being overproduced in cells, without obvious DNA damage.
73. Radman, M. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis. *Basic Life Sci.* **5A**, 355–367 (1975).
74. Echols, H. SOS functions, cancer and inducible evolution. *Cell* **25**, 1–2 (1981).
75. Schaaper, R. M. & Radman, M. The extreme mutator effect of *Escherichia coli* mutD5 results from saturation of mismatch repair by excessive DNA replication errors. *EMBO J.* **8**, 3511–3516 (1989).
76. Foster, P. L. Nonadaptive mutations occur in the F′ episome during adaptive mutation conditions in *Escherichia coli*. *J. Bacteriol.* **179**, 1550–1554 (1997).
77. Godoy, V. G., Gizatullin, F. S. & Fox, M. S. Some features of the mutability of bacteria during nonlethal selection. *Genetics* **154**, 49–59 (2000).
78. Rosenberg, S. M., Thulin, C. & Harris, R. S. Transient and heritable mutators in adaptive evolution in the lab and in nature. *Genetics* **148**, 1559–1566 (1998).
79. Bull, H. J., McKenzie, G. J., Hastings, P. J. & Rosenberg, S. M. Evidence that stationary-phase hypermutation in the *E. coli* chromosome is promoted by recombination. *Genetics* **154**, 1427–1437 (2000).
80. Bull, H. J., McKenzie, G. J., Hastings, P. J. & Rosenberg, S. M. The contribution of transiently hypermutable cells to mutation in stationary phase. *Genetics* **156**, 925–926 (2000).
81. Bull, H. J., Lombardo, M.-J. & Rosenberg, S. M. Stationary-phase mutation in the bacterial chromosome: recombination protein and DNA polymerase IV dependence. *Proc. Natl Acad. Sci. USA* (2001, in the press).
The first demonstration of recombination-protein (RecA and RuvC)-dependent and DNA-pol IV-dependent mutation in the *Escherichia coli* chromosome, in stationary phase. This shows that Rec- and pol IV-dependent stationary-phase mutation can affect the main bacterial genome, as well as plasmid-borne genes.
82. Cairns, J. The contribution of transiently hypermutable cells to mutation in stationary phase. *Genetics* **156**, 923–926 (2000).
83. Rosenberg, S. M. Mutation for survival. *Curr. Opin. Genet. Dev.* **7**, 829–834 (1997).
84. Foster, P. L. & Trimarchi, J. M. Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation. *Proc. Natl Acad. Sci. USA* **92**, 5487–5490 (1995).
85. Radicella, J. P., Park, P. U. & Fox, M. S. Adaptive mutation in *Escherichia coli*: a role for conjugation. *Science* **268**, 418–420 (1995).
86. Galitski, T. & Roth, J. R. Evidence that F′ transfer replication underlies apparent adaptive mutation. *Science* **268**, 421–423 (1995).
87. Foster, P. L. & Trimarchi, J. M. Conjugation is not required for adaptive reversion of an episomal frameshift mutation in *Escherichia coli*. *J. Bacteriol.* **177**, 6670–6671 (1995).
88. Rosenberg, S. M., Harris, R. S. & Torkelson, J. Molecular handles on adaptive mutation. *Mol. Microbiol.* **18**, 185–189 (1995).
89. Lombardo, M.-J., Torkelson, J., Bull, H. J., McKenzie, G. J. & Rosenberg, S. M. Mechanisms of genome-wide hypermutation in stationary phase. *Ann. NY Acad. Sci.* **870**, 275–289 (1999).
90. Yarmolinsky, M. B. Programmed cell death in bacterial populations. *Science* **267**, 836–837 (1995).
91. Firth, N., Ippen-Ihler, K. & Skurray, R. H. in Escherichia coli *and* Salmonella*: Cellular and Molecular Biology* (eds Neidhardt, F. C. *et al.*) 2377–2401 (ASM, Washington, DC, 1996).
92. Whoriskey, S. K., Nghiem, V.-H., Leong, P.-M., Masson, J.-M. & Miller, J. H. Genetic rearrangement and gene amplification in *Escherichia coli*: DNA sequences at the junctures of amplified gene fusions. *Genes Dev.* **1**, 227–237 (1987).
93. Tlsty, T. D., Margolin, B. H. & Lum, K. Differences in the rates of gene amplification in nontumorigenic and tumorigenic cell lines as measured by Luria–Delbrück fluctuation analysis. *Proc. Natl Acad. Sci. USA* **86**, 9441–9445 (1989).
94. Pipiras, E., Coquelle, A., Bieth, A. & Debatisse, M. Interstitial deletions and intrachromosomal amplification initiated from a double-strand break targeted to a mammalian chromosome. *EMBO J.* **17**, 325–333 (1998).
Demonstration that DNA double-stranded breaks provoke amplification in mammalian cells (as it is implied that they do in *Escherichia coli*, reference 13).
95. Ono, S. *Evolution by Gene Duplication* (Springer, Berlin and New York, 1970).
96. Jasin, M. in *DNA Damage and Repair. 3. Advances from Phage to Humans* (eds Nickoloff, J. A. & Hoekstra, M. F.) 207–235 (Humana, Inc., Totowa, New Jersey, 1998).
97. Brodeur, G. M. & Hogarty, M. D. in *The Genetic Basis of Human Cancer* (eds Vogelstein, B. & Kinzler, K. W.) 161–172 (McGraw–Hill, New York, 1998).
98. Coquelle, A., Pipiras, E., Toledo, F., Buttin, G. & Debatisse, M. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. *Cell* **89**, 215–225 (1997).
99. Deitsch, K. W., Moxon, E. R. & Wellems, T. E. Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. *Microbiol. Mol. Biol. Rev.* **61**, 281–293 (1997).
Review of the importance of simple repeat tracts and frameshift mutation in the expression of genes used in pathogenesis.
100. Saunders, N. J. *et al.* Repeat-associated phase variable genes in the complete genome sequence of *Neisseria meningitidis* strain MC58. *Mol. Microbiol.* **37**, 207–215 (2000).
101. Martinez, J. L. & Baquero, F. Mutation frequencies and antibiotic resistance. *Antimicrob. Agents Chemother.* **44**, 1771–1777 (2000).
An excellent review of mutation-based routes to antibiotic resistance, in which it is pointed out that even antibiotics that kill cells can be non-lethal, and thus capable of stimulating stress-promoted mutations at lower concentrations.
102. Zambrano, M. M. & Kolter, R. GASPing for life in stationary phase. *Cell* **86**, 181–184 (1996).
103. LeClerc, J. E., Li, B., Payne, W. L. & Cebula, T. A. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* **274**, 1208–1211 (1996).
104. Denamur, E. *et al.* Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. *Cell* **103**, 711–721 (2000).
105. Cashel, M., Gentry, D. R., Hernandez, V. J. & Vinella, D. in Escherichia coli *and* Salmonella: *Cellular and Molecular Biology* (eds Neidhardt, F. C. *et al.*) 1458–1496 (ASM, Washington, DC, 1996).
106. Datta, A. & Jinks-Robertson, S. Association of increased spontaneous mutation rates with high levels of transcription in yeast. *Science* **268**, 1616–1619 (1995).
107. Davis, B. D. Transcriptional bias: a non-Lamarckian mechanism for substrate-induced mutations. *Proc. Natl Acad. Sci. USA* **86**, 5005–5009 (1989).
108. Strauss, B. S. The origin of point mutations in human tumor cells. *Cancer Res.* **52**, 249–253 (1992).
One of the first suggestions that adaptive mutation mechanisms are apt models for mutations that lead to cancer. See also references 37, 110 and 111.
109. Hall, B. G. Adaptive mutations in *Escherichia coli* as a model for the multiple mutational origins of cancer. *Proc. Natl Acad. Sci. USA* **92**, 5669–5673 (1995).
110. Finch, C. E. & Goodman, M. F. Relevance of 'adaptive' mutations arising in non-dividing cells of microorganisms to age-related changes in mutant phenotypes of neurons. *Trends Neurosci.* **20**, 501–507 (1997).
111. Drake, J. W. Spontaneous mutation. *Annu. Rev. Genet.* **25**, 125–146 (1991).
112. Walter, C. A., Intano, G. W., McCarrey, J. R., McMahan, C. A. & Walter, R. B. Mutation frequency declines in spermatogenesis in young mice but increases in old mice. *Proc. Natl Acad. Sci. USA* **95**, 10015–10019 (1998).
113. Swanson, W. J., Yang, Z., Wolfner, M. F. & Aquadro, C. F. Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals. *Proc. Natl Acad. Sci. USA* **98**, 2509–2514 (2001).
The discovery of hypervariable genes that encode the coat proteins of mammalian eggs. Perhaps this, and the cognate hypervariable genes that encode sperm surface proteins, are targets for mammalian DinB1, a mutator DNA polymerase expressed in the germ line.
114. Harris, R. S., Kong, Q. & Maizels, N. Somatic hypermutation and the three R's: repair, replication and recombination. *Mutat. Res.* **436**, 157–178 (1999).
115. Jacobs, H., Rajewsky, K., Fukita, Y. & Bross, L. Indirect and direct evidence for DNA double-strand breaks in hypermutating immunoglobulin genes. *Phil. Trans. R. Soc. Lond.* B **356**, 119–125 (2001).
116. McDonald, J. P. *et al.* DNA polymerase iota and related Rad30-like enzymes. *Phil. Trans. R. Soc. Lond.* B **356**, 53–60 (2001).
117. True, H. L. & Lindquist, S. L. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* **407**, 477–483 (2000).
118. Delbrück, M. *Cold Spring Harbor Symp. Quant. Biol.* **11**, 154 (1946).
119. Stewart, F. M., Gordon, D. M. & Levin, B. R. Fluctuation analysis: the probability distribution of the number of mutants under different conditions. *Genetics* **124** (1990).
120. Higgins, N. P., Kato, K. & Strauss, B. J. A model for replication repair in mammalian cells *Mol. Biol.* **101**, 417–425 (1976).
121. Louarn, J.-M., Louarn, J., Francois, V. & Patte, J. Analysis and possible role of hyperrecombination in the terminus region of the *Escherichia coli* chromosome. *J. Bacteriol.* **173**, 5096–5104 (1991).
122. Seigneur, M., Bidnenko, V., Ehrlich, S. D. & Michel, B. RuvAB acts at arrested replication forks. *Cell* **95**, 419–430 (1998).
123. Powell, S. C. & Wartell, R. M. Different characteristics distinguish early versus late arising adaptive mutations in *Escherichia coli* FC40. *Mutat. Res.* **473**, 219–228 (2001).
124. Akerlund, T., Nordstrom, K. & Bernander, R. Analysis of cell size and DNA content in exponentially growing and stationary-phase batch cultures of *Escherichia coli*. *J. Bacteriol.* **177**, 6791–6797 (1995).
125. Anderson, R. P. & Roth, J. R. Spontaneous tandem duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (rrn) cistrons. *Proc. Natl Acad. Sci. USA* **78**, 3113–3117 (1981).
126. Zan, H. *et al.* The translesion DNA polymerase zeta plays a major role in Ig and *bcl-6* somatic hypermutation. *Immunity* **14**, 643–653 (2001).
127. Rogozin, I. B., Pavlov, Y. I., Bebenek, K., Matsuda, T. & Kunkel, T. A. Somatic mutation hotspots correlate with DNA polymerase eta error spectrum. *Nature Immunol.* **2**, 530–536 (2001).
128. Zeng, X. *et al.* DNA polymerase eta is an A–T mutator in somatic hypermutation of immunoglobulin variable genes. *Nature Immunol.* **2**, 537–541 (2001).
129. Chicurel, M. Can organisms speed their own evolution? *Science* **292**, 1824–1827 (2001).

Acknowledgements
I apologize to my many colleagues whose relevant papers I could not cite owing to space limitations. Thanks to P. J. Hastings, S. Jinks-Robertson, M.-J. Lombardo, N. Maizels and B. Wright for discussions; C. Dorman and J. Strathern for communicating unpublished results; and W. Arber, J. Drake, E. Friedberg, P. J. Hastings, M.-J. Lombardo, G. J. McKenzie, J. Ninio, J. Petrosino and E. Witkin for comments on the manuscript. Supported by grants from the National Institutes of Health (USA).