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Adaptive mutation: implications for evolution

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Summary

Adaptive mutation is defined as a process that, during nonlethal selections, produces mutations that relieve the selective pressure whether or not other, nonselected mutations are also produced. Examples of adaptive mutation or related phenomena have been reported in bacteria and yeast but not yet outside of microorganisms. A decade of research on adaptive mutation has revealed mechanisms that may increase mutation rates under adverse conditions. This article focuses on mechanisms that produce adaptive mutations in one strain of *Escherichia coli*, FC40. These mechanisms include recombination-induced DNA replication, the placement of genes on a conjugal plasmid, and a transient mutator state. The implications of these various phenomena for adaptive evolution in microorganisms are discussed.

Introduction

It has long been assumed that the force driving evolution is natural selection, not the creation of genetic variants, because the rate of mutation was thought to be constant and unaffected by circumstances (although Darwin himself did not believe this). But, a controversial paper published by John Cairns and his colleagues in 1988(1) challenged traditional thinking about spontaneous mutation. In that paper, Cairns et al. presented evidence that mutations arise in non-dividing, nutritionally deprived cells of *Escherichia coli*, apparently in response to selective pressure. The Lamarckian idea that these mutations were “directed” by the selective conditions was based on three observations: (1) mutations arose among nonproliferating cells after selection was applied, (2) the presence of the selective agent was required for the mutations, and (3) mutations that were not selected did not appear during selection. Cairns et al. gave several examples illustrating these points, and other cases quickly appeared in print. (2–7) Only some of these cases were further investigated, and, in general, one or more of the above criteria has proved not to be true or to be explainable by other causes (reviewed in Refs 8–10). The original hypothesis of “directed” mutation, therefore, has not been supported. Nevertheless much subsequent research has shown that mutation rates can vary, and that they increase during certain stresses such as nutritional deprivation. The phenomenon has come to be called “adaptive mutation”, by which is meant a process that during nonlethal selection produces mutations that relieve the selective pressure, whether or not other, nonselected mutations are also produced.(10) It remains to be seen whether this occurs via an evolved mechanism, or because the cells are simply unable to maintain the integrity of their DNA repair systems.

Examples of adaptive mutation or related phenomena have been reported in the bacteria *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, *Pseudomonas* sp. and *Clostridium* sp., and, in the eukaryotic microbial species, *Saccharomyces cerevisiae* and *Candida albicans* (Refs 8–16). Whether the phenomenon exists outside of microorganisms is not known, but it seems unlikely to contribute to the evolution of organisms that have their

germ lines protected from the environment. Mutations occurring in the nondividing somatic cells of higher organisms, however, could give rise to disorders such as cancer.(9,17,18)

In the last decade, most research on adaptive mutation has utilized *Escherichia coli* strain FC40. (6) Consequently, more is known about the mechanisms that produce adaptive mutations in this strain than in any other. Although the results cannot necessarily be extrapolated to other cases, FC40 provides examples of the kinds of mechanisms that can give rise to adaptive mutations. In this essay, I describe these mechanisms and discuss their possible evolutionary consequences.

Characteristics of strain FC40

Although FC40(6) is not the strain of *E. coli* originally described in Cairns et al.,(1) it has proved attractive for studies of adaptive mutation because of its high rate of mutation during selection. References to the work of several groups using this strain can be found in Ref. 10. The strain is deleted for the *lac* operon on its chromosome but carries this operon on the F episome, a low-copy conjugal plasmid. The strain cannot utilize lactose (Lac^-) because of a +1 frameshift mutation that inactivates the *lacZ* gene. During nonselective growth FC40 has a mutation rate to lactose utilization (Lac^+) of about 1 per 10^9 cells per generation; when plated on lactose minimal medium, Lac^+ revertants arise at a rate of about 1 per 10^9 cells per hour, and continue to do so at this rate for several days (Fig. 1). Yet, during such a selective regime, in a carefully conducted experiment the total number of background (Lac^-) cells does not change by more than 20%, and there is no evidence of cell death or turnover.(19) In effect, there is a genuine and specific increase in the number of mutational events that produce Lac^+ revertants.

The Lac^+ mutations that occur during lactose selection are distinguished from those that occur during nonselected growth by type and by genetic requirements. While insertions and deletions of various types giving a Lac^+ phenotype occur during nonselective growth, the adaptive Lac^+ mutations are almost exclusively – 1 base pair deletions in runs of iterated bases. Furthermore, unlike mutation during nonselective growth, adaptive Lac^+ mutation requires recombination functions, specifically *E. coli*'s pathway for double-strand end repair. In addition, the high rate of adaptive Lac^+ mutation requires that the *lac* allele be on the F⁺ episome and that conjugal functions be expressed, although actual conjugation is not required. In contrast, when the same *lac* allele is in its normal position on the chromosome, the adaptive mutation rate to Lac^+ falls 100-fold, and the mutations are no longer dependent on recombination or conjugal functions.(10)

Although adaptive mutation in FC40 might be considered a laboratory artefact, it has several potentially important lessons to teach us. First, recombination-dependent mutation might be an important source of spontaneous mutations when cells are not actively replicating their genomes. Second, error-prone polymerases might increase genetic variation under stress. Third, conjugal plasmids might be important in the evolution and horizontal transfer of genes. These subjects are discussed below.

Recombination-dependent mutation

Recombination and DNA replication

With the exception of certain genomic rearrangements, the creation of a mutation requires DNA synthesis. Although the role of recombination in adaptive mutation in FC40 may be indirect, the simplest hypothesis, which is supported by much current research, is that recombination initiates the DNA synthesis that creates the mutations. It is well established that DNA replication and recombination are intimately connected in bacteriophage T4,(20) but the

generality of this connection has only recently been recognized. Successful chromosome replication requires recombination because replication forks frequently stall and, in *E. coli* and many other organisms, the stalled forks are restarted through recombinational processes.(21–23) If the replication fork encounters a single-strand break (a nick), the fork will collapse and be repaired via the double-strand end repair pathway (in *E. coli*, the RecBCD pathway). If the replication fork encounters a DNA lesion, the fork will disassemble and reassemble downstream of the lesion; the resulting gap is filled by recombination with the other template strand (in *E. coli*, the RecF pathway). Both pathways depend on RecA recombinase. The importance of these processes in the normal life of a cell is indicated by the debilitating effect of simultaneous loss of recombination and DNA repair capacity.

While it is thus easy to understand why successful chromosomal replication might require recombination, it is not so obvious why recombination might require DNA replication. The most suggestive genetic evidence is that cells with null mutations in the gene *priA*, which encodes a protein required for certain forms of DNA replication (e.g., that of bacteriophage Φ X174), are partially defective for homologous recombination.(24) Recently, physical evidence has been obtained that DNA replication is, in fact, induced by recombinational double-strand end repair in *E. coli*.(25)

Smith hypothesized that, during reciprocal exchange, an endless chain of recombination events would be initiated unless replication primed by invading 3' ends produced double-stranded circles.(26) Some replication, however, is also required for nonreciprocal recombination. Perhaps the partial dependence of recombination on replication reflects that recombination evolved to repair stalled replication forks and the recombination and replication enzymes are evolutionarily linked.

The replication that is primed by recombination is assumed to be error-free. But is it? Demerec found that mutations arose in *Salmonella typhimurium* at high frequencies during generalized bacteriophage transduction with the recipient strain's own DNA, and that this took place even when the donor DNA was deleted for the target gene.(27) Similarly, a high frequency of mutations in linked genes was observed during transformation of cells with homologous DNA;(28,29) in *Anacystis nidulans*, it was shown that this mutation occurred only when recombination took place between the linked genes.(28) The simplest hypothesis to explain these results is that recombination of incoming DNA primes DNA synthesis and that this synthesis produces mutations. An old phenomenon called induced-stable DNA replication (iSDR), which we now understand to be DNA synthesis primed by 3' single-stranded ends invading homologous double-stranded DNA,(30) was reported to be error prone.(31) More recently, double-strand breaks induced in *Saccharomyces cerevisiae* were shown to produce mutations in nearby genes.(32) In this system base substitutions, but not frameshifts, were dependent on REV3,(33) which encodes the catalytic subunit of DNA polymerase ζ , one of the newly discovered error-prone polymerases(34) (see below).

The role of recombination in adaptive mutation

In light of the above studies, it seems likely that DNA synthesis primed by an invading 3' end during recombination produces the adaptive Lac⁺ mutations in FC40. The Lac[−] allele is on the conjugal plasmid, F', which carries a special origin for conjugal transfer called *oriT*. Nicking at *oriT* by the nickase, TraI, occurs even when the cells are not mating.(35,36) This nicking can initiate recombination.(37) F also has vegetative origins from which normal replication forks are initiated. Because the Lac[−] allele is slightly leaky, some DNA replication may occur during lactose selection. The best current hypothesis for adaptive mutation in FC40 that accounts for all the genetic requirements is that a replication fork initiated at a vegetative origin encounters the persistent nick at *oriT* and collapses. The broken arm of the replication fork is repaired by double-strand end repair, and de novo DNA synthesis initiated by the

recombination intermediate produces the Lac⁺ mutations (Fig. 2).(38,39) The fact that adaptive mutations are eliminated in *priA* mutants(10) supports this hypothesis.

The role of error-prone polymerases in adaptive mutation of FC40

Because the cells are not actively growing, the production of mutations by the recombination-dependent mechanism becomes dominant during lactose selection. But, even so, the mutation rate observed is surprisingly high, equivalent (in mutations per cell per hour) to that observed when the cells are growing exponentially. It seems unlikely that DNA replication and turnover in Lac⁻ cells during lactose selection is equal to that occurring during exponential growth. Thus, it appears that the DNA synthesis that gives rise to the mutations is inherently error-prone, refractory to repair, or both.

Recently, a new class of error-prone DNA polymerases has been discovered in bacteria and higher organisms, including humans.(34) Because, at least in *E. coli*, these polymerases are induced by DNA damage (as part of the 'SOS response' reviewed in Ref. 40) and possibly by other stresses, it has been hypothesized that they exist to increase variation in a population during stress.(41) Alternatively, the error-prone polymerases may exist solely to replicate damaged DNA, and their error proneness may be an inevitable consequence of this requirement.(42)

It is now known that *E. coli* has at least five DNA polymerases, three of which are induced as part of the SOS response. Two of the inducible polymerases, Pol IV and V, are error-prone, while one, Pol II, is accurate.(43) In FC40, Pol IV normally accounts for 50% of the adaptive Lac⁺ mutations, but for many more in the absence of Pol II.(44) This means that Pol II and Pol IV are in competition, and Pol II normally limits Pol IV to about 20% of its mutagenic potential. The remaining mutations may be due to Pol III (the replicative polymerase) or to another polymerase. Thus, at least three and possibly more DNA polymerases appear to compete for the 3' ends of recombination intermediates. The adaptive mutation rate is, at least in part, determined by the relative levels of these polymerases. Since induction of these polymerases is under control of the SOS system, which can be induced in nutritionally deprived cells (see below), this is a way that stress can increase mutation rates, as was envisioned many years ago. (45)

Evolutionary significance of recombination-induced mutation

The recombination-dependent mutational mechanism is particularly active on F (see below), but can be expected to occur whenever a nick is encountered during DNA replication. As discussed above, accumulating evidence indicates that: (1) recombination is constantly active in both proliferating and non-proliferating cells, (2) that recombination initiates DNA synthesis, and (3) that this synthesis can lead to mutations. Thus, recombination not only rearranges existing alleles, it can also create new genetic variants via associated DNA synthesis. This may not be a major source of variation in growing organisms when other mutational mechanisms are active, but might become significant in static populations. In *E. coli*, many of the components of this system—the recombinase, the specialized polymerases and the enzymes that help resolve recombination intermediates—are induced as part of the SOS response to DNA damage. SOS genes are also induced in aging colonies(46) and at the end of growth in complex medium.(47) Similar inducible systems may exist in other organisms, allowing limited transient mutator states to be active when genetic variability may be advantageous.

Strain FC40 detects frameshift mutations, and it might be argued that these are not particularly significant in evolution (although they are important in switching certain antigen-determinant genes on and off, see Ref. 48). The mutagenic mechanism illustrated in Fig. 2, however, is not specific for frameshifts; all types of mutation may be produced, particularly when the error-

prone polymerases are induced. I hypothesize that both of *E. coli*'s error-prone polymerases can participate in double-strand end repair, but since Pol IV is more readily induced than Pol V,(49) it dominates the response. Although Pol IV tends to make frameshifts,(49) both Pol IV and Pol V also make base substitutions.(50,51) Base substitutions are considered to be more evolutionarily significant than frameshifts because these mutations are more likely to alter, not eliminate, protein function.

Conjugal plasmids

Our original belief that in FC40 only Lac⁺ mutations occurred during lactose selection was based on a failure to find mutations in a control gene (giving rifampicin resistance) among non-reverted Lac⁻ cells.(19) The Lac⁻ allele is on the conjugal plasmid, F, however, and the target for mutations to rifampicin resistance, *rpoB*, is on the chromosome. When a suitable target was placed close to the Lac⁻ allele on F, non-selected mutations in this gene were readily observed during lactose selection. These mutations occurred at about the same rate and by the same recombination-dependent mechanism as did the Lac⁺ mutations.(52) Mutations at another locus on F also occurs at a high rate.(53) Thus, genes carried on F appear to be highly mutable.

F recombines with the chromosome via insertional elements, creating various Hfr strains. When it excises it can carry with it large segments of the chromosome, creating various F's. The genes on these F's actively recombine with their homologues on the chromosome.(37,54) Thus, we might consider F to be an evolution machine—it can pick up genes from the chromosome, expose them to a high mutation rate, transfer the genes into new hosts, and recombine the new alleles back onto the chromosome. (It should be noted that comparative mutation rates of genes on F's other than the one carried by FC40 have not been determined).

As discussed above, nicking at *oriT* is likely to be the important initiating event for mutation. TraI, the enzyme that nicks the DNA, is encoded by one of the genes of the *tra* operon, which encodes the proteins required for conjugal transfer. Laboratory strains of *E. coli* are constitutive for *tra* expression because the original F⁺ strain isolated by Leder-berg, from which all laboratory Fs are derived, has an insertion in *finO*, a gene that encodes a negative regulator of the *tra* operon. No natural F's have been found with this insertion.(55) Other than the presence of F⁻ cells, however, we do not know what factors may influence *tra* expression in nature. One indication that *tra* expression is responsive to environmental conditions is that *traY*, which encodes a positive regulator of the *tra* operon, is under control of two different transcriptional regulators, ArcA and CpxR, both of which are part of two-component sensor-response systems.(56,57) If expression of the *tra* operon is increased by starvation or other stresses, then nicking by TraI would be more frequent, resulting in a higher rate of recombination-dependent mutation of genes in *cis* to *oriT*. This is a second way, in addition to induction of error-prone polymerases, that stress could increase mutation rates but, in this case, only of genes carried on the F episome.

How common are F plasmids in nature? Although long believed to be rare, recent work revealed that 21% of natural isolates of *E. coli* carry either F or related conjugal plasmids.(55) Significantly, these plasmids showed evidence of extensive recombination as well as recent horizontal transfer among diverse *E. coli* strains, and even between *E. coli* and *S. typhimurium*.(55,58) Thus, F may play an important and previously unappreciated role in determining bacterial population structure, in horizontal gene transfer, and in the appearance of new genetic variants.(55,58,59)

Transient mutation

How important are transient mutators?

An early hypothesis to explain adaptive mutation was that a subpopulation of cells under selection enters into a state of high mutation (the hypermutable state). If a cell in this state achieves a mutation that relieves the selective pressure, it begins to grow and exits the hypermutable state. If it does not achieve a successful mutation, it dies. This process would appear to be adaptive because cells that carry only irrelevant mutations are eliminated from the population. A cell that achieved success, however, would carry non-selected mutations along with the mutation that allowed it to grow.(7)

Although this hypothesis is no longer required to explain adaptive mutation, the basic idea lives on. In support of the hypothesis, several studies have found that selected mutants carry non-selected mutations at higher than expected frequencies.(5,7,52,53,60,61) Yet these cells are not stable mutators, i.e. they have normal mutation rates after they start to grow. If we accept, that a population under selection is heterogeneous for mutation rates, how important are the high mutators and what is the cause of their elevated mutation rate?

On the basis of theoretical considerations, Ninio concluded that transient mutators would produce only 10% of the single mutations but more the 95% of simultaneous double mutations that arise in a population.(62) Using an algebraic model developed by Cairns, we were able to calculate these values from real data and obtained results remarkably similar to Ninio's. In a population of FC40 cells under lactose selection, we found that about 0.1% of the cells experience a mutation rate that is 200-fold higher than normal. These cells give rise to about 12% of the singly mutant cells, 97% of the doubly mutant cells, and essentially all the cells carrying three more mutations (Fig. 3). We also found that the proportion of Lac⁺ cells that carry a second mutation increased linearly with time, suggesting that the high mutators do not die as a result of their mutational load, at least during the 5 days of a typical experiment.(53)

Thus, during selection, hypermutators account for only a minority of single mutations, including the mutations to Lac⁺. This result can be easily understood without resorting to equations by noting that the frequency of a given mutant class was 10-fold higher in Lac⁺ cells carrying a second non-selected mutation than in Lac⁺ cells without a second mutation.(53) A very similar value, 20-fold, was obtained in a separate study.(60) Therefore, cells that produce double mutations (the high mutators) have much higher mutation rates (because they have a higher frequency of a third mutation) than cells that produce single mutations. This means that most single mutations do not arise from the hypermutator class, but from cells that have a lower mutation rate. The simplest hypothesis is that all the cells in the population are mutating; a minority has a very high mutation rate and gives rise to virtually all multiple mutants, and the majority has a lower mutation rate and gives rise to most of the single mutants (Fig. 3).

What causes transient mutation?

Ninio reasoned that a cell would become a transient mutator if it failed to inherit the requisite number of DNA repair proteins.(62) Subsequently it was shown that the levels of the proteins responsible for methyl-directed mismatch repair in *E. coli* do, indeed, decline in stationary phase cells,(63,64) although most cells retain sufficient mismatch repair activity for the limited DNA synthesis that occurs.(65,66) We tested Ninio's hypothesis and found that, during lactose selection, a mismatch repair defect raised the frequency of non-selected mutations among Lac⁻ cells but not among Lac⁺ cells. Thus, defects in mismatch repair raise the mutation rate of normal cells, but apparently do not raise the mutation rate of the cells that are transient mutators. This result is consistent with the hypothesis that mismatch repair is already deficient or saturated in the hypermutating subpopulation but not in the normal majority.(53)

Another possible cause of transient mutation is the creation of defective repair and replication enzymes by mistranscription or, more likely, mistranslation.(62) Changes in the proofreading subunit of DNA polymerase III could have a large effect because the mutant protein would be incorporated into the replication complex and give rise to a tract of mutations. Interestingly, mutator strains have been isolated that are due to tRNAs that insert the wrong amino acid at certain codons, and a probable target is the proofreading subunit of Pol III.(67,68)

A third possible cause of transient mutation in a subpopulation is induction of SOS functions. About 0.1% of the cells in a stationary-phase culture of FC40 are filamentous, an indicator of SOS induction.(69) As mentioned above, SOS induction results in increased levels of the error-prone polymerases Pol IV and Pol V. While Pol IV accounts for only half of the adaptive Lac⁺ mutations, it might account for all of the mutations in hypermutators. In support of this idea, preliminary data suggest that the frequency of double mutations is lower in cells deficient in Pol IV than in wild-type cells (S. L. Leugers and P.L.F., unpublished data). Similarly, an increase in multiple mutations was observed in genetic backgrounds in which SOS is chronically induced.(70)

The significance of high mutation rates for evolution

An analysis by Drake(71) revealed that, among DNA-based microorganisms, spontaneous mutation rates per genome are remarkably constant (see article by Sniegowski et al., this issue). Although genome sizes varied over four orders of magnitude, the number of mutations per genome varied only 2.5-fold. Thus, mutation rates have evolved so that each organism makes, on average, about one mutation every 300 rounds of replication.

Because most mutations are deleterious, one would assume that mutation rates would be as low as possible consistent with the cost (in terms of energy and time) of accurate DNA replication and repair. In *E. coli*, heritable mutators appear at frequencies of one in 10⁵, reflecting the 30 or so genes that can give a mutator phenotype. Yet, 1–3% of natural isolates of *E. coli* and *S. typhimurium* are heritable mutators, implying mutators can be advantageous in natural environments (reviewed in Ref. 72). Heritable mutators also are enriched by certain artificial selection regimens.(73–76)

The enrichment of mutators during severe or prolonged selection does not mean that there is a positive selection for mutators per se, but that mutators can increase the speed at which adaptive mutations occur. There is a problem, however. The mutator allele also gives rise to deleterious mutations, and it will ‘hitchhike’ to fixation along with any advantageous mutation.(77) In an asexual population, there is no easy way to unlink the adaptive changes from the mutator allele. Hence, the mutators that take over the population must either revert to normal or mutate themselves to extinction. Several mathematical models have been developed to deal with this problem.(78,79) In general, these models predict that small populations and rapidly changing conditions favor mutators. In addition, mutators may have more of an advantage in highly adapted populations in which advantageous mutations are rare.(80) Indeed, in such populations (which, presumably, include *E. coli* growing in the laboratory) significant adaptation may require more than one mutation.

The transient mutator state described above is a more advantageous way of generating genetic diversity than the random appearance of heritable mutators. Since only a small portion of the population enters the mutator state, most cells have a normal mutation rate. So, if advantageous mutations are common (because the cells are poorly adapted to the environment, or because the current problem is easy to solve), the cells with normal mutation rates achieve success and carry no extra mutational burden. However, if advantageous mutations are rare, or if more than one mutation is needed, the hypermutating cells will achieve success. Of course, they will be

burdened with extra mutations. Yet, because the hypermutable state is transient, their mutation rates return to normal while they enjoy their success.

The transient mutator state resembles another poorly understood phenomenon, natural competence for DNA uptake. In *Bacillus subtilis*, for example, such competence is transient, is a response to nutritional deprivation, and involves only a small proportion (10%) of the population. Most of the cells do not become competent, but instead make long-lived spores. Hence, like transient mutation, natural competence appears to be a mechanism to allow some members of the population to increase their genetic variability while the majority of the population stays safely static (reviewed in Ref. 81).

It is possible that transient mutation is simply a pathological state induced by nutritional deprivation. The more interesting possibility, however, is that it is an adaptation. If so, how could such a trait as hypermutation (or competence) evolve, given that it is often, perhaps usually, disadvantageous to the cell expressing it? As with other behavioral traits, the genes determining hypermutation may be expressed rarely, but on occasion have such a large advantage that they are preserved. In addition, it is useful to remember that a population of bacteria in a given environment is likely to be clonal. So, although the individuals compete for resources, they are also closely related; if any member of the group survives, most of the genes in the population will as well.

A phenotype may have evolved, however, because it confers some particular general advantage rather than because it very occasionally confers some other benefit. For example, transient hypermutators may exist because there is an evolutionary advantage for cells that save energy when conditions are disadvantageous to growth by downregulating certain pathways for DNA repair (F. Taddei, personal communication). But, once in a while such phenotypes will yield a big pay-off by generating a useful mutation, allowing the cells to start proliferating. Once in place, such a mechanism might be preserved by virtue of this payoff and its relatively small cost.

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Figure 1.

The accumulation of Lac⁺ revertants of FC40 during incubation on lactose minimal medium plates. Left axis, circles: the number of Lac⁺ colonies per 10⁸ cells; right axis, triangles: the number of Lac⁻ cells on the plate. Adapted from Ref ⁶.

Figure 2.

A model for recombination-dependent mutation. **A,B:** Collapse of the replication fork at *oriT*; **C,D:** RecABCD-catalyzed recombination; **E:** restoration of the replication fork; **F:** translocation of the Holliday junction by RuvAB and RecG; resolution of the Holliday junction by RuvC is not shown. Dashed lines are newly synthesized DNA; * at *oriT* indicates TraI. The polarity of the DNA has been inverted between B and C. Adapted from Ref. ¹⁰.

Figure 3.

Venn diagram illustrating the role of transient mutators in adaptive mutation. Adapted from Ref. ¹⁰.