Adaptive Mutation: Has the Unicorn Landed?

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ABSTRACT

Reversion of an episomal Lac$^-$ allele during lactose selection has been studied as a model for adaptive mutation. Although recent results show that the mutations that arise during selection are not "adaptive" in the original sense, the mutagenic mechanism that produces these mutations may nonetheless be of evolutionary significance. In addition, a transient mutational state induced in a subpopulation of starving cells could provide a species with a mechanism for adaptive evolution.

"Adaptive mutation is a strategy, not a mechanism."
Jan Drake (1991, personal communication)

IN 1988, John Cairns and his collaborators published an article entitled "The Origin of Mutants" (Cairns *et al.* 1988) that has changed our thinking about how spontaneous mutations arise. Drawing upon their own and others' results, they argued that mutations arise in nondividing bacterial cells subjected to nonlethal selective pressure. Additional evidence suggested that only selected mutations, not deleterious or neutral mutations, appeared in a population during selection. Obviously, this phenomenon would have profound implications for evolution and for carcinogenesis. In 1989, Cairns and I began a collaboration to further study the phenomenon, which was dubbed "directed mutation" by the editors of *Nature* and "a unicorn in the garden" by Stahl (1988).

Early in the project, we established that the mutational process was not "directed" toward specific targets (*i.e.*, there was no reverse information flow) (Foster and Cairns 1992), and we renamed the phenomenon "adaptive mutation" (Foster 1993). We then pursued the alternative hypothesis that during selection a random mutational process affecting the whole genome might occur; the process would be adaptive if the variants (or the cells bearing them) were transient unless or until a variant arose that allowed the cell to grow (Cairns *et al.* 1988; Stahl 1988; Boe 1990; Hall 1990). Although less efficient than a directed mechanism, "trial and error" would have equivalent implications. With such a mechanism, a population could increase its genetic variability under stress yet maintain its genes more or less intact.

In the intervening years, many examples of mutation in nondividing cells have been published (reviewed in Foster 1993; also see Taddei *et al.* 1995; Bridges 1996; Galitski and Roth 1996; Hall 1997; Kasak *et al.* 1997; Reddy and Gowrishankar 1997). Not all are examples of adaptive mutation. In some cases, it has been shown that the mutations can also arise in the absence of selection (*e.g.*, when the cells are merely starving) (Mittler and Lenski 1990; Mittler and Lenski 1992; Foster and Cairns 1994; Maenhaut-Michel and Shapiro 1994; Sniegowski 1995). In the case of one of the best-studied examples of adaptive mutation, reversion to lactose utilization (Lac$^+$) in *Escherichia coli* strain FC40, we now know that nonselected mutations also arise in the Lac$^-$ population during lactose selection (Foster 1997). However, among selected Lac$^+$ clones the frequency of nonselected mutations appears to be higher than among the population at large (Foster 1997; Torkelson *et al.* 1997). These results imply that in a population of stressed cells, a subpopulation undergoes some form of transient mutation, as originally suggested by Hall (1990). However, contrary to Hall's hypothesis, the cells bearing nonselected mutations do not necessarily disappear (Foster 1997). Nonetheless, this mechanism would provide a population with the means to evolve adaptively when confronted with adverse conditions.

Cairns has contributed to this issue a history of adaptive mutation and a discussion of its relevance for carcinogenesis (Cairns 1998). Here, I focus on the mechanism of adaptive mutation in FC40 and its relevance for evolution. This particular mechanism does not necessarily underlie other cases of adaptive mutation (see the references cited in the previous paragraph for examples of other mechanisms). As Jan Drake perceived (quoted above), the phenomenon we observe as "adaptive mutation" is a strategy to overcome adversity, and cells may have diverse ways of achieving this goal.

Because nonselected mutations arise and persist in the population during selection, a stress-associated general mutational state, strictly speaking, does not meet the original definition of adaptive mutation. However, here I will continue to call the selected mutations "adaptive" to distinguish them from mutations occurring during nonselective growth and from nonselected mutations occurring during selection. This meaning of "adaptive muta-

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tion" is the same as that used by evolutionists to distinguish beneficial from neutral or deleterious mutations.

The mechanism of adaptive mutation to Lac$^+$ in FC40: To study adaptive mutation, Cairns and I chose a strain of *Escherichia coli* that cannot utilize lactose (Lac$^-$) because of a +1 base pair (bp) frameshift mutation affecting the *lacZ* gene (Calos and Miller 1981; Miller 1985). For ease of genetic manipulation, the Lac$^-$ allele is carried on an F′ episome, which turned out to be crucial to the mutagenic mechanism. This strain, FC40, readily reverts to lactose utilization (Lac$^+$) when lactose is its sole energy and carbon source (Cairns and Foster 1991). Because of FC40's vigorous mutational response (about one Lac$^+$ revertant per 10^7 cells per day), it was possible to eliminate many trivial explanations for the results (such as growth during lactose selection) and to explore the mechanism by which mutations arise in the nondividing cells.

In FC40, the mechanism of mutation to Lac$^+$ during lactose selection is different from the mechanism of mutation to Lac$^+$ during nonselective growth: (1) The spectrum of Lac$^+$ mutations that arise during lactose selection is distinct. Although a variety of deletions, duplications, and frameshifts revert the Lac$^-$ allele during growth, adaptive Lac$^+$ mutations consist almost exclusively of −1-bp frameshifts in runs of iterated bases (Foster and Trimarchi 1994; Rosenberg *et al.* 1994). (2) Adaptive, but not growth-dependent, reversion to Lac$^+$ requires recombination functions, specifically the activities of the *recA-recBCD* pathway (Cairns and Foster 1991; Foster 1993; Harris *et al.* 1994). (3) But certain recombination functions have different roles in adaptive mutation than they do in normal recombination. *E. coli*'s two enzyme systems for the branch migration of recombination intermediates, RuvAB and RecG, both contribute to normal recombination (West 1996), but RuvAB promotes and RecG opposes adaptive Lac$^+$ mutation (Foster *et al.* 1996; Harris *et al.* 1996). (4) The high level of adaptive reversion to Lac$^+$ in FC40 requires that the Lac$^-$ allele be on the episome; if the same allele is at its normal position on the chromosome, adaptive reversion to Lac$^+$ falls about 100-fold and is no longer *recA*-dependent (Foster and Trimarchi 1995a; Radicella *et al.* 1995). In addition, the high rate of adaptive Lac$^+$ mutation on the episome requires that one or more conjugal functions be expressed (Foster and Trimarchi 1995a; Galitski and Roth 1995); however, actual conjugation is not required (Foster and Trimarchi 1995a,b).

In two respects, the adaptive Lac$^+$ mutations are similar to normal growth-dependent mutations: (1) Adaptive Lac$^+$ mutations are produced by DNA polymerase III, *E. coli*'s replicative polymerase (Foster *et al.* 1995; Harris *et al.* 1997a). DNA polymerase II also replicates DNA, particularly in stationary-phase cells, but it produces few errors (Escarceller *et al.* 1994; Foster *et al.* 1995; Rangarajan *et al.* 1997). (2) The methyl-directed mismatch repair (MMR) pathway, which corrects mismatches in hemi-methylated DNA in favor of the methylated strand (Modrich and Lahue 1996), corrects about 99% of the errors that could lead to adaptive Lac$^+$ mutations (Foster and Cairns 1992; Foster *et al.* 1996; Harris *et al.* 1997a). The residual mutation rate can be reduced two- to fivefold by overproducing components of the MMR pathway (Foster *et al.* 1995; Foster *et al.* 1996; Harris *et al.* 1997b).

A model for the mechanism of adaptive mutation to Lac$^+$ in FC40: Nicking at the conjugal origin *oriT* is known to initiate recombination (Carter *et al.* 1992); thus, the initiating event for adaptive mutation to Lac$^+$ in FC40 is likely to be a nick at *oriT*. Previous studies have indicated that conjugation can be mutagenic (Kunz and Glickman 1983; Christensen *et al.* 1985), and it is possible that conjugal replication initiated by nicking produces the Lac$^+$ mutations (Foster and Trimarchi 1995a; Galitski and Roth 1995; Radicella *et al.* 1995). However, the involvement of RecBCD implicates a double-strand break (DSB), the loading point for this enzyme (Kowalczykowski *et al.* 1994), and it is not obvious how a DSB would be created during conjugal replication. In addition, the unusual effects of the branch migration enzymes suggest that the recombination functions have special roles in adaptive mutation to Lac$^+$.

Kuzminov (1995) proposed that the DSB is created when a replication fork initiated at one of the episome's vegetative origins collapses at the nick at *oriT* (Figure 1, A–C). The exonuclease and helicase activities of RecBCD then create an invasive 3′ end that initiates recombination (Figure 1D). After both strands have invaded homologous duplex DNA (of the same or another episome), the replication fork is restored and replication resumes (Figure 2A). Replication errors produced at this point are in hemi-methylated DNA and are correctable by MMR (Figure 2B). But the new fork differs from a normal fork in that it is accompanied by a four-stranded recombination intermediate (a Holliday junction). Migration of the Holliday junction toward the fork creates a tract of doubly unmethylated DNA in which polymerase errors will be randomly repaired by MMR. This tract will thus contain a higher-than-normal number of mutations (Figure 2, C and D, left). Migration of the junction away from the fork (Figure 2, C and D, right) or resolution of the Holliday junction before DNA synthesis begins (Figure 3) preserves the hemi-methylated state of the DNA, allowing polymerase errors to be correctly repaired.

The opposite effects of the branch migration enzymes on adaptive mutation to Lac$^+$ are accommodated by assuming that RuvAB and RecG promote migration of the Holliday junction away from and toward the replication fork, respectively (Figure 2), or that RecG resolves the Holliday junction before replication resumes (Figure 3) (Foster *et al.* 1996). Both possibilities are consistent with biochemical evidence showing that RuvAB and RecG have different interactions with recombination intermediates (Whitby *et al.* 1993; Whitby and Lloyd 1995).

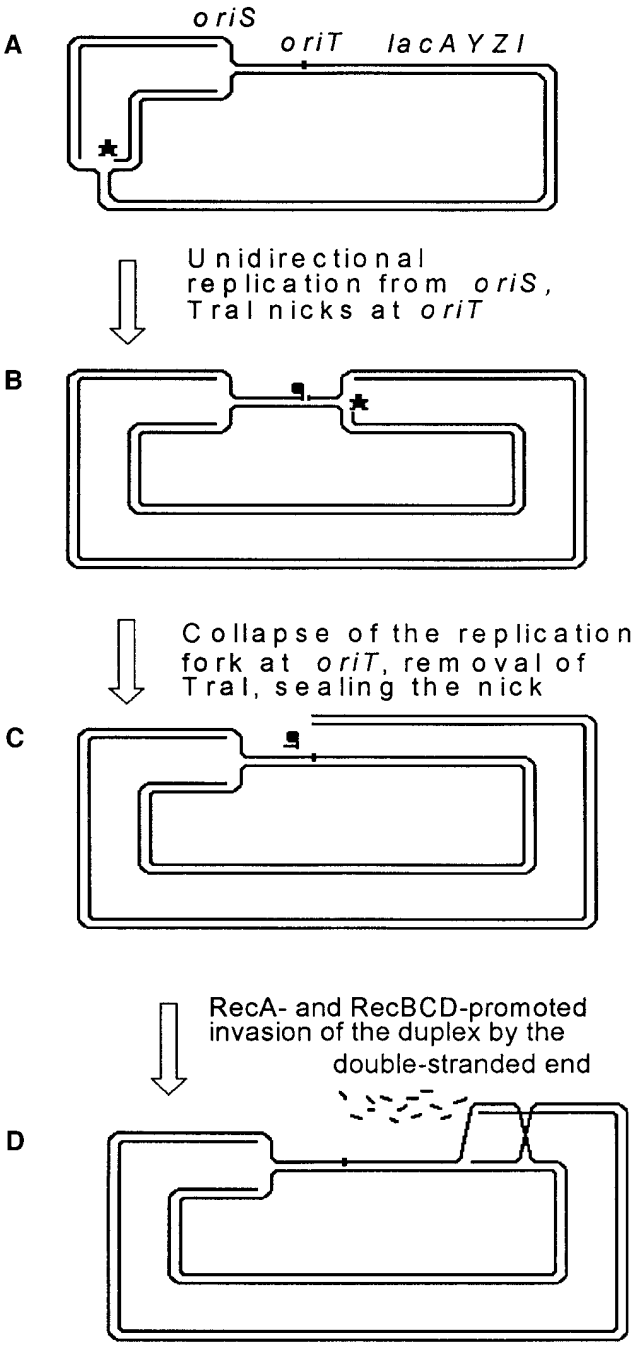

Figure 1.—Initiation of double-strand end invasion by collapse of the replication fork at *oriT*. A star marks the 3′ end of the counterclockwise moving fork. TraI is indicated by a flag. Reprinted from Foster *et al.* (1996).

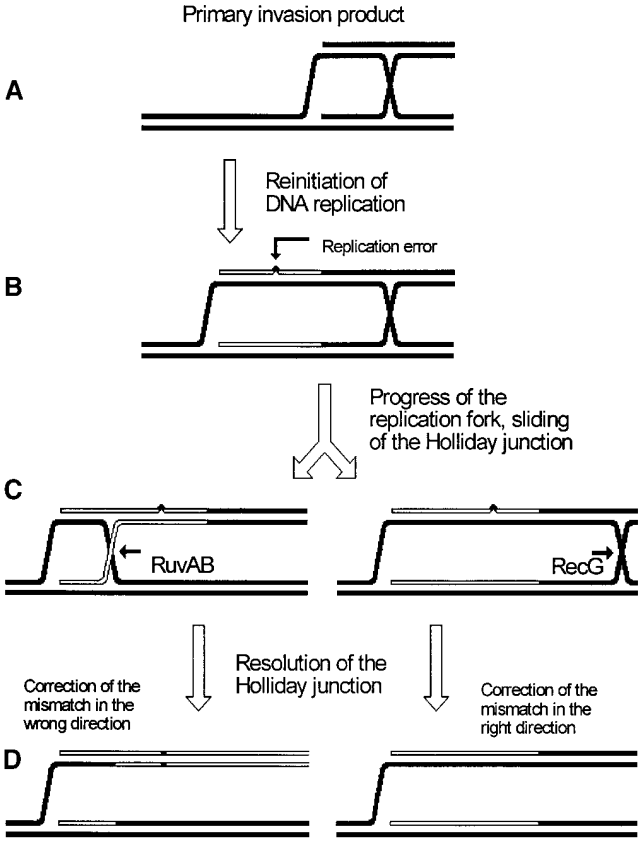

Figure 2.—Re-establishment of the replication fork and translocation of the Holliday junction in opposite directions by RuvAB and RecG. Newly synthesized DNA is indicated by open lines, and template DNA by closed lines. The 3′ end is the lower strand in each case. Reprinted from Foster *et al.* (1996).

Several other models are possible (Foster *et al.* 1996; Harris *et al.* 1996), but this one is the most parsimonious. The model also accounts for the fact that whereas MMR is active in nutritionally deprived cells, the mutational spectrum bears the mark of MMR deficiency (see below).

The significance of *recA*-dependent mutation: The *recA*-dependent mechanism in FC40 is just one of the ways by which mutations could arise in nondividing cells. In nondividing or slowly dividing cells, the mutability of a gene may depend critically on its proximity to a site where DNA synthesis is active. Thus, what is special about the episome may be simply the frequency and persistence of the nick at *oriT*. But similar events could occur on the chromosome. Spontaneous or damage-induced nicks in the chromosomal DNA will likewise lead to a collapsed replication fork, triggering a *recA-recBCD*-dependent recombination event that establishes a new replication fork (Kuzminov 1995). If the subsequent synthesis is error-prone or poorly corrected, or if the recombination is iterative, genes near a frequent nick site will accumulate mutations. Independently of a moving replication fork, DSBs themselves could initiate DNA synthesis by the same recombinational mechanism if a homologue is present. DSBs could occur by breakage of the other strand at a nick or by DNA damage. In addition, at certain sites in the chromosome, called *oriM*'s, frequent (possibly enzymatically induced) DSBs have been proposed to initiate *recA*-dependent "stable" DNA-replication (SDR) (Kogoma 1997). (The relationship between DSB repair, SDR, and adaptive mutation is complicated by their different genetic requirements, but all three appear to involve the same recombination events leading to DNA synthesis.) At other sites on the chromo-

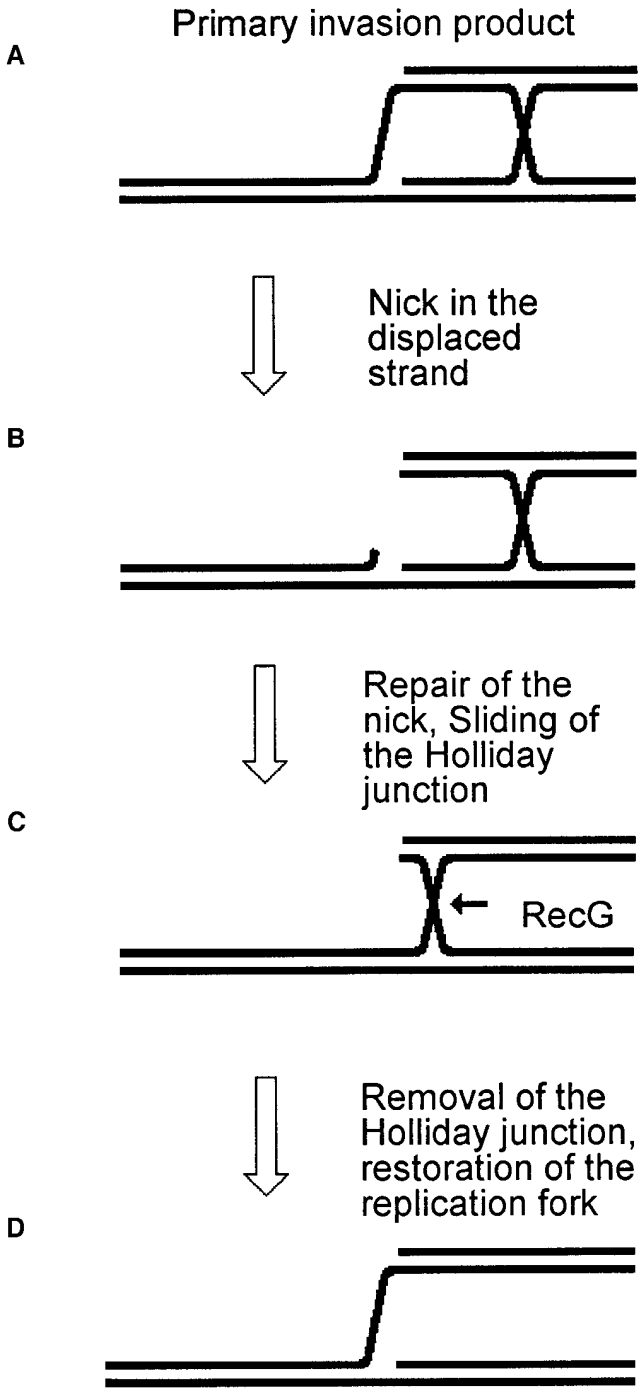

Figure 3.—Removal of the Holliday junction by RecG and subsequent re-establishment of the replication fork. The 3′ end is the lower strand in each case. Reprinted from Foster *et al.* (1996).

some, called *oriK*'s, another form of SDR is initiated by transcription in RNaseH mutants (Kogoma 1997). Thus, if RNaseH levels are low or inhibited in nutritionally deprived cells, *oriK*'s would be active.

The occurrence of nonselected mutations during selection: We inferred that mutation to Lac$^+$ in FC40 was adaptive because Lac$^+$ mutations did not arise when cells were starved in the absence of lactose (Cairns and Foster 1991). In addition, nonselected mutations giving a Rif^R phenotype (mutations in the chromosomal gene *rpoB*) did not appear in the Lac$^-$ population during lactose selection (Foster 1994). As mentioned above, the Lac$^-$ allele in FC40 is carried on an F′ episome, which raised the possibility that the mutational process was confined to the episome. If chromosomal loci were not involved, this would give the appearance that the mutations were adaptive (Foster and Trimarchi 1995a; Galitski and Roth 1995; Radicella *et al.* 1995). This hypothesis was tested with tetracycline-sensitive (Tet^S) Tn*10* elements close to the *lac* operon on the episome. The two mutants characterized carried +1-bp frameshifts in runs of G:C bps in *tetA*, and thus were very similar to the Lac$^-$ allele. In contrast to the chromosomal *rpoB* gene, the mutant *tetA* alleles readily reverted to Tet^R when the cells were under selection to become Lac$^+$. The Tet^R mutations accumulated at nearly the same rate and occurred by the same *recA*-dependent mechanism as the Lac$^+$ mutations (Foster 1997). That the Tet^R mutations appeared and persisted in the Lac$^-$ population disproved the hypotheses that nonselected mutations are necessarily transitory or that the cells (or episomes) bearing them are necessarily eliminated from the population. Because neither Lac$^+$ nor Tet^R mutations arose if lactose was not present (*i.e.*, when the cells were merely starving), the role of lactose is apparently to provide enough energy (because the Lac$^-$ allele is "leaky") for DNA replication and recombination even though the cells are not actively dividing. (For other examples of adaptive reversion of "leaky" alleles, see Jayaraman 1995; Galitski and Roth 1996.)

Lac$^+$ Tet^R double mutants also arose in the Lac$^-$ Tet^S population at a frequency about 50-fold higher than would be predicted from the individual mutation rates to Lac$^+$ and Tet^R (Foster 1997). Torkelson *et al.* (1997) also found that Lac$^+$ revertants of FC40 carried second, nonselected mutations at several additional loci on the episome, on a plasmid, and on the chromosome. Thus, the mutational process is not confined to the episome. Again, the frequency of nonselected mutations among Lac$^+$ clones was 50-fold or more higher than in the Lac$^-$ population. However, in contrast to the episomal Lac$^+$ and Tet^R mutations (Foster 1997), there is no evidence published to date that these other nonselected mutations are produced by the same mechanism as the Lac$^+$ mutations. But, because the two classes of mutation preferentially appear in the same cells, there must be some rate-limiting process affecting both selected and nonselected mutations.

The transient mutation model: In two previous studies, a higher-than-expected frequency of nonselected mutations had been found among selected clones (Boe 1990; Hall 1990). This result is a specific prediction of the "hypermutable state model" (Hall 1990), although it is also implicitly predicted by all the "trial and error"

models for adaptive mutation [because when a cell starts growing, the genetic variant that allowed it to grow will necessarily be retained, but other variants present in the cell at that moment will also have some probability of being preserved (Foster 1992)]. But the frequencies at which double mutants were found in these various studies, if accurate, compel a mutating minority. To account for the occurrence and persistence of nonselected mutations in the population at large, Hall's model can be modified as follows: In a starving or otherwise stressed population, a small proportion of the cells enter into a state of increased mutation. It is not known what triggers this state, but because Lac^+ mutations accumulate at a constant rate (Cairns and Foster 1991; Foster 1994), the number of mutating cells must be constant with time. These cells give rise to mutants at random, but the rates at which different loci are mutated differ. Differential mutability may be due to position (genes on the episome may be readily mutated because of their proximity to a site of frequent recombination) and the nature of the target (frameshifts may occur more frequently than base substitutions). However, cells carrying a selected mutation (*e.g.*, the Lac^+ cells) will be far more likely to carry second, nonselected mutations than cells without the selected mutation (the Lac^- cells). This is because the mutating minority is generating Lac^+ mutants at a higher rate than the nonmutating majority, and the Lac^+ mutant population will be enriched for the cells that have passed through a period of mutation.

This hypothesis, also discussed by Bridges (1997), is supported by results from Miller's laboratory (Mao *et al.* 1997), demonstrating the ease at which heritable mutators can be enriched in a selected population. Heritable mutators appear to be only minor contributors to the double mutant population in the experiments discussed above (Torkelson *et al.* 1997), implying that the state of mutation is usually transient. Thus, a population under stress could temporarily increase its mutation rate in a minority, increasing the chance that a lucky variant will arise, but the majority of cells would remain unchanged in the event a mutation was not needed (Cairns 1998).

Possible mechanisms for transient mutation: Cairns (1998) has discussed the possibility that the mutating minority may consist of cells that have sustained a transcriptional or translational error leading to a faulty DNA polymerase or DNA repair enzyme (Ninio 1991; Boe 1992; Cairns 1998). Here are two additional possibilities: the down-regulation of an error-correcting pathway or the up-regulation of an error-producing pathway.

The spectrum of adaptive mutations in FC40 is typical of polymerase errors that are not corrected by MMR (Foster and Trimarchi 1994; Rosenberg *et al.* 1994). This fact immediately suggested that the mutations occur because MMR levels decline in starving cells, as originally suggested by Stahl (1988). But does MMR decline? Biochemical evidence indicated that the levels of two MMR proteins, MutH and (particularly) MutS, drop sharply in starving cells (Feng *et al.* 1996). But genetic evidence indicated otherwise—defects in MMR dramatically increase adaptive mutation in FC40 and other strains, showing that MMR is active in nutritionally deprived cells (Boe 1990; Foster and Cairns 1992; Jayaraman 1992; Harris *et al.* 1997a; Reddy and Gowrishankar 1997). This apparent contradiction has been resolved by new biochemical data showing that the decline in MMR proteins is considerably less than previously reported (Harris *et al.* 1997b). Thus, if a reduction of MMR is responsible for transient mutation, only a subpopulation of cells are affected. Consistent with this idea, overproduction of MMR proteins reduces adaptive mutation in FC40 two- to fivefold (Foster *et al.* 1995; Foster *et al.* 1996; Harris *et al.* 1997b). However, MMR proteins in excess may have pathological consequences, for example, by inhibiting the branch migration that is required for recombination (Foster *et al.* 1996; Modrich and Lahue 1996; Zahrt and Maloy 1997). Nonetheless, a role for MMR in adaptive mutation is appealing and is supported by recent evidence that certain MutS-defective tumor cells become mutators only when grown to high densities (Richards *et al.* 1997).

An alternative hypothesis can account for all the results in FC40. As mentioned above, with the exception of Lac^+ and Tet^R mutations on the episome (Foster 1997), there is no evidence published to date that the selected and nonselected mutations are produced by the same mechanism, but only that some rate-limiting process affects them both. Although adaptive Lac^+ mutations do not require SOS-induced error-prone DNA synthesis, they do require certain genes, such as *recA* and *ruvAB* (Cairns and Foster 1991; Foster *et al.* 1996), that are repressed by LexA, the SOS repressor (Friedberg *et al.* 1995). The SOS response is induced in old colonies (Taddei *et al.* 1995), and recombination is stimulated by the presence of F′ factors (Syvanen *et al.* 1986). About 0.1% of the cells in a stationary-phase culture of FC40 are filaments (W. A. Rosche and P. L. Foster, unpublished results), a phenotype of LexA-derepression. If Lac^+ mutants are drawn preferentially from the pool of SOS-induced cells because these cells have induced levels of RecA and RuvAB, Lac^+ clones could carry nonselected mutations that result not only from a *recA*-dependent mechanism but also from SOS-dependent error-prone repair.

Which, if any, of these hypotheses is correct remains to be seen. At the outset of our studies we naïvely assumed that a universal mechanism would underlie adaptive mutation. But genetics proved us wrong as it became apparent that many cases of adaptive mutation do not involve the *recA*-dependent mechanism that is active in FC40. Now it is tempting to consider transient mutation to be the unique "cause" of adaptive mutation. However,

this idea will also probably turn out to be naïve. Transient mutation, if it is real, may itself be due to many causes. So at this juncture it would be wise to again recall Jan Drake's comment that is quoted at the start of this article.

The evolutionary significance of adaptive mutation: The research reviewed here has several implications for evolution. First, a recombination-dependent mechanism could be an important source of spontaneous mutations in *E. coli* and other organisms. Recombination events are often accompanied by tracts of DNA synthesis; if these are associated with a high probability of mutations, as indicated by previous studies (Demerec 1963; Strathern *et al.* 1995), then recombination can increase variation not only by rearranging existing alleles but also by creating new ones. Second, the *recA*-dependent mutagenic mechanism is highly active on the F episome. Conjugal plasmids are common among natural isolates of bacteria (Clewell 1993). On an evolutionary time scale, F and related plasmids frequently recombine and are passed among the major groups of *E. coli* and *Salmonella enterica* (Boyd *et al.* 1996; Boyd and Hartl 1997). Because F can recombine with the bacterial chromosome, it can pick up and transfer chromosomal genes (Holloway and Low 1996), which would then be exposed to the episomal mutation rate and be free to diverge from their chromosomal copies. Thus, the mutational mechanism on the episome may be important in the evolution of species that carry and exchange conjugal plasmids. Third, as discussed above, if nutritionally deprived cells enter into a state of transient mutation, this could provide a mechanism for adaptive evolution under adverse conditions.

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