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The Origin of Adaptive Mutants: Random or Nonrandom?

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Abstract. Several recent reports have claimed that adaptive mutants in bacteria and yeast are induced by selective conditions. The results of these reports suggest that mutants can arise nonrandomly with respect to fitness, contrary to what has been widely accepted. In several cases that have received careful experimental reexamination, however, the detection of seemingly nonrandom mutation has been explained as an experimental artifact. In the remaining cases, there is no evidence to suggest that cells have the capacity to direct or choose which genetic variants will arise. Instead, current models propose processes by which genetic variants persist as mutations only if they enable cell growth and DNA replication. Most of these models are apparently contradicted by experimental data. One model, the hypermutable state model, has recently received limited circumstantial support. However, in this model the *origin* of adaptive mutants is random; the apparent nonrandomness of mutation is merely a consequence of natural selection. The critical distinction between the origin of genetic variation (mutation) and the possible consequence of that variation (selection) has been neglected by proponents of directed mutation.

Key words: Adaptive mutation — Directed mutation — Natural selection — Selection-induced mutation

Introduction

> *In short, mutations are accidents, and accidents will happen.* (Sturtevant 1937)

It is a fundamental tenet of modern biology that mutations are accidents—that is, that they occur at random with respect to their immediate fitness consequences. Firm experimental support for the randomness of mutation was provided by three classical experiments in bacterial genetics: the fluctuation test (Luria and Delbrück 1943), replica plating (Lederberg and Lederberg 1952), and sib selection (Cavalli-Sforza and Lederberg 1956). Some recent investigations in bacteria and in yeast, however, claim that mutation is nonrandom when cells are incubated on a medium that permits growth only if certain mutations occur (Cairns et al. 1988; Hall 1988, 1990, 1991, 1992; Cairns and Foster 1991; Steele and Jinks-Robertson 1992). The validity of this conclusion has been questioned in a number of cases, from both experimental and theoretical perspectives (Charlesworth et al. 1988; Lenski 1989; Lenski et al. 1989; Symonds 1989; Mittler and Lenski 1990, 1992; Stewart et al. 1990; Benson et al. 1991; Smith 1992; Lenski and Mittler 1993; MacPhee 1993a,b; Dijkmans et al. 1994). Nonetheless, some have concluded that nonrandom mutation is a real phenomenon whose underlying cellular basis awaits discovery (Drake 1991b; Stahl 1992; Foster 1993). Studies of apparently directed mutation may indeed reveal novel genetic and physiological phenomena (Foster 1992). However, the possibility that mutations arise in a goal-directed manner—that they might not be accidents—has not been demonstrated.

Semantic Issues

There has been confusion over what term should be given to nonrandom mutation. At the root of this confusion is failure to distinguish between the *origin of adaptive mutants* and the *selective consequences* of their oc-

currence. This is the crux of the entire controversy: Are adaptive mutants specifically caused to arise by selective circumstances, or are they merely selectively recovered? Two of the terms in recent use, "selection-induced mutation" and "adaptive mutation," are confusing in this respect. "Selection-induced mutation" (e.g., Hall 1994) stands on its head the accepted meaning of "selection" (MacPhee 1993a,b). Selection is not a physical causative agent but a consequence of variation for fitness. Selection itself is incapable of inducing adaptive mutants; the question is whether the physical selective environment somehow causes adaptive mutants to arise nonrandomly. "Adaptive mutations" and "adaptive mutation" (Foster 1993) are even less satisfactory terms. The qualifier "adaptive" does not mean "nonrandomly induced." Mutations that arise randomly can be adaptive in the accepted sense of conferring high fitness; even mutations that arise randomly and are initially deleterious can later become adaptive. In a fluctuating environment, a high rate of random mutation can be adaptive (Leigh 1970, 1973; Gillespie 1981a,b; Ishii et al. 1989); hence, "adaptive mutation" could equally well be used to refer to rapid random mutation as an evolutionary accommodation to extreme environmental unpredictability.

The term "directed mutation" is used to refer to nonrandom mutation throughout this paper. In its strongest sense, "directed mutation" implies an instructional mechanism that specifies favorable genetic changes (Lenski and Mittler 1993). Since no evidence of such a mechanism has been forthcoming (see below), proponents of nonrandom mutation have backed away from initial implications (e.g., Cairns et al. 1988) that mutations might be truly directed in this manner. As used in the present paper, "directed mutation" need not imply specific instructional mechanisms. Instead, it refers generally to the possibility that "bacteria, in stationary phase, have some way of producing (or selectively retaining) only the most appropriate mutations" (Cairns et al. 1988).

The Nature of the Evidence for Directed Mutation

Many types of directed mutation are seemingly possible, including point mutations, frame shifts, suppressors, and excisions of mobile genetic elements (Foster 1993); perhaps there are multiple mechanisms for directed mutation, or perhaps misleading experimental results can be obtained in a variety of genetic contexts. It is noteworthy that all of the evidence for directed mutation has been obtained from a single type of experiment, in which cells are incubated in a medium that allows growth only if certain mutation(s) occur. Directed mutation is invoked if the rate at which such beneficial mutations occur appears to rise only under selective circumstances, while rates of mutation at loci unrelated to the selection remain unaffected.

Lenski and Mittler (1993) have identified several effects that can produce a false appearance of directed mutation in such experiments. Each of these effects can result in nondirected changes in observed mutation rates of many orders of magnitude, and the bias due to more than one effect operating at once can be multiplicative. These effects include: (1) nonspecific increases in mutation rate due to uncontrolled environmental factors (e.g., a mutagenic effect of starvation unrelated to the selective agent); (2) overestimation of mutation rates due to growth of mutants or intermediates; (3) underestimation of mutants arising on "control" media (containing no usable carbon) or of mutants unrelated to the selection, due to death of mutants or their failure to grow into detectable colonies as quickly as expected; and (4) growth of the progenitor population of cells at risk for mutation on the selective medium due to contaminating substrates, leaky phenotypes, or crossfeeding. Below, I discuss several examples of apparently directed mutation that are likely to be artifactual due to the operation of one or more of these effects.

Alternatives to Directed Mutation

Lac(Ara)$^+$ *Mutations in* Escherichia coli *MCS2*

In *E. coli* strain MCS2 (Shapiro 1984), part of the *ara* operon (including a regulatory region) has been joined to structural genes from the *lac* operon by DNA from a Mu prophage. With this prophage intact, MCS2 is incapable of utilizing either lactose or arabinose for growth. Upon excision of the prophage in an appropriate reading frame, however, MCS2 is phenotypically Lac(Ara)$^+$; it is capable of utilizing lactose with arabinose as an inducer. Lac(Ara)$^+$ mutants are extremely rare ($<10^{-9}$) in MCS2 cultures growing on glucose or glycerol (Shapiro 1984; Cairns et al. 1989; Mittler and Lenski 1990). Shapiro (1984), however, observed large numbers of Lac(Ara)$^+$ mutants in MCS2 cultures starved for several days on a solid minimal medium containing only lactose and arabinose as potential carbon sources. Cairns et al. (1988) conducted further experiments in liquid media with and without these sugars that led them to conclude that Lac(Ara)$^+$ mutants arise in a directed manner, i.e., that Mu excises *only* when cells are starving in the presence of lactose and arabinose.

Mittler and Lenski (1990) replicated the observations of Shapiro (1984) and Cairns et al. (1988), but showed that Lac(Ara)$^+$ mutants also arise in starving cultures in solid and liquid media when no sugars are present. This result suggested that Lac(Ara)$^+$ mutants are not directed by the presence of lactose and arabinose, but instead are induced by starvation alone. Although the numbers of Lac(Ara)$^+$ mutants that Mittler and Lenski (1990) observed on plates with lactose and arabinose were still

somewhat higher than those on plates with no sugars, this difference was traceable to an increase in the number of progenitor Lac(Ara)$^-$ cells susceptible to mutation on plates with lactose and arabinose as a result of cross-feeding by Lac(Ara)$^+$ cells. With this cryptic growth taken into account, the inferred rates of mutation to Lac(Ara)$^+$ on media with and without the selective sugars were statistically indistinguishable.

Critics of the Mittler and Lenski (1990) results (Shapiro and Leach 1990; Foster 1993) noted that, in order to detect Lac(Ara)$^+$ mutants in MCS2 plate cultures starving without sugars, Mittler and Lenski directly exposed these cultures to lactose and arabinose (this was done by spraying starved plate cultures with a solution of arabinose and lactose); this procedure did not eliminate the possibility that starvation simply prepares certain Lac(Ara)$^-$ cells to undergo rapid directed mutation to Lac(Ara)$^+$ upon exposure to lactose and arabinose. Mittler and Lenski did show, however, that the frequency of Lac(Ara)$^+$ mutants detected in a nonselectively starved MCS2 culture is stable when that culture is regrown in glucose; this result does not support the existence during starvation of unstable intermediates that rapidly convert to the Lac(Ara)$^+$ phenotype upon exposure to lactose and arabinose. Further studies using the classical techniques of sib selection (Maenhaut-Michel and Shapiro, in press), fluctuation analysis (Foster and Cairns, in press), and replica plating (P. Sniegowski, in preparation) have solidly confirmed Mittler and Lenski's (1990) conclusion that mutation to Lac(Ara)$^+$ is induced by starvation alone.

The Mu case illustrates the inappropriateness of comparing mutation rates between cells starving in the presence of a selective agent (in this case, the combination of lactose and arabinose) and cells growing in its absence. Such a comparison fails to control for a nonspecific effect of starvation on mutation rate. In addition, this case illustrates the potential for cryptic growth of nonmutant progenitor cells in apparently ''starving'' cultures on selective medium. In the following two cases, cryptic growth of mutational intermediates, rather than progenitors, contributes to a false appearance of directed mutation.

Double Mutants in the bgl *Operon*

Hall (1988) studied an *E. coli* K12 strain in which two mutations in the *bgl* operon are apparently required for growth on salicin: excision of an insertion sequence, *IS*150, from a structural gene, *bglF,* and a mutation in a regulatory region, *bglR.* The *IS*150 insertion in this strain is extremely stable in growing cultures, such that excisions are almost never detected (Hall 1988). However, Hall detected large numbers of salicin-utilizing double mutants after prolonged incubation of cells on a solid medium supplemented with salicin as the only available sugar. Hall claimed that these double mutants were arising as a result of directed excision of *IS*150 followed by random mutation in *bglR.* Hall presented evidence that an isolated excision mutant clone was incapable of growth on salicin without the second mutation in *bglR* and argued that *IS*150 excisions were, therefore, occurring only when this would open the way to a beneficial second mutation that would allow resumed growth. Mittler and Lenski (1992), however, showed that many excision mutants (including the single one tested by Hall) are capable of some growth on salicin; it was, therefore, not necessary to invoke anticipatory directed mutation to explain the initial increase in numbers of excision-mutant intermediates. Furthermore, this selective enrichment of excision-mutant intermediates increased the expected number of fully Sal$^+$ double mutants by many orders of magnitude, such that there was no need to invoke directed mutation at all in Hall's system.

Subsequent to the report of Mittler and Lenski (1992), Hall published a study acknowledging that excision mutants are capable of some growth on salicin without the second mutation in *bglR* and that such growth can explain his previous results in the *bgl* system without the need to invoke directed mutation (Hall 1994). In this new study, however, Hall claims that *IS*150 excision is nonetheless directed in a genetic background in which no other mutations are required for full utilization of salicin. To date, this new claim has not been challenged experimentally. The results of Mittler and Lenski (1992), however, suggested that, contrary to Hall's (1988) report, *IS*150 excision mutants do arise in cultures starved without salicin; thus, it is possible that Hall's new claim of directed mutation in the *bgl* operon can be explained as the consequence of a nondirected increase in *IS*150 excision during starvation. Further experiments utilizing classical genetic techniques such as replica plating or sib selection (i.e., similar to those applied in the case of Mu excisions described above) may provide critical evidence on this question.

trpA trpB *Double Revertants*

Hall (1990, 1991) has also claimed that the double reversion of a *trpA trpB* mutant of *E. coli* in the absence of tryptophan is ''selection-induced'' in that *trpA*$^+$ *trpB*$^+$ cells arise at far higher rates than expected from the product of the reversion rates of single *trpA* and *trpB* mutants in similar circumstances. However, single mutant (i.e., *trpA*$^+$ *trpB* or *trpA trpB*$^+$) intermediates might be capable of growth on indole, a tryptophan precursor which can accumulate in medium without tryptophan as a result of direct excretion or breakdown of excreted indoleglycerol phosphate (Foster 1992). As in the *bgl* case, such selective enrichment of single mutant intermediates could lead to a much higher rate of recovery of double mutants from starving *trpA trpB* populations without the need to invoke directed mutation. Indeed,

further experiments by Hall (1993) revealed substantial growth of *trpA trpB*$^+$ single revertant cells in mixed culture with *trpA trpB* cells on selective medium. Hall now appears to admit, albeit reluctantly, that enrichment of *trpA trpB*$^+$ intermediates may account for the *trpA*$^+$ *trpB*$^+$ double revertants observed (Hall 1993).

Biased Recovery of Dex$^+$ Mutants

Benson et al. (Benson 1988; Benson et al. 1988, 1991) examined the possibility of directed mutation in an *E. coli* strain that is phenotypically Dex$^-$; it lacks the LamB outer membrane protein and so cannot grow on large maltodextrins. Dex$^+$ phenotypes in this strain can arise as a result of mutations in genes for two other membrane proteins, OmpC and OmpF. Benson et al. (1988) found that when Dex$^-$ populations are starved on a medium containing only maltodextrins as a potential carbon source, OmpF$^+$ mutants apparently occur at a much higher frequency than OmpC$^+$ mutants, as though directed mutation is taking place at the *ompF* locus (Benson 1988). Further investigation, however, revealed that OmpF$^+$ mutants overgrow their Dex$^-$ progenitors much more quickly than OmpC$^+$ mutants, which leads to a bias in the recovery, rather than the occurrence, of the OmpF$^+$ mutation (Benson et al. 1991).

Fluctuation Analysis: Reversion to Leucine Prototrophy in Salmonella typhimurium

"Poisson-like" distributions of mutants among selective plates, markedly different from the Luria-Delbrück distributions expected if mutants arise only before plating (Luria and Delbrück 1943), can be expected in fluctuation tests if some mutants arise by directed mutation (Cairns et al. 1988). However, Poisson-like distributions can also result from various violations of the assumptions of Luria and Delbrück (e.g., if mutants in preplating cultures grow more slowly than progenitor cells); hence, the appearance of Poisson-like distributions in fluctuation tests does not necessarily indicate directed mutation (Koch 1982; Charlesworth et al. 1988; Tessman 1988; Lenski et al. 1989; Stewart et al. 1990; Lenski and Mittler 1993; Stewart 1994). A recent study (Dijkmans et al. 1994) has documented yet another means by which Poisson-like distributions can occur without directed mutation. Dijkmans et al. (1994) used fluctuation analysis to examine reversion to prototropy in a Leu$^-$ strain of *S. typhimurium* and observed Poisson-like distribution patterns of Leu$^+$ mutants on selective plates. The preplating growth rates of Leu$^+$ mutants in their study were similar to that of the progenitor Leu$^-$ population, and this seemed to rule out a conventional explanation in favor of directed mutation. However, many Leu$^+$ clones consisted of cells that were ten- to 100-fold larger than nonmutant cells. Since the transition from nonmutant Leu$^-$ to much larger Leu$^+$ mutant cells is likely to involve an initial delay in cell division as mutant daughter cells increase in size, Dijkmans et al. (1994) postulated that this delay could be responsible for the observed Poisson-like distribution of seemingly directed, late-arising Leu$^+$ mutants on selective plates. In support of this, they found normal cell sizes within mutant colonies that arose early on selective plates and followed a Luria-Delbrück distribution.

Other Claims

A number of other claims of directed mutation have not received experimental challenge (e.g., Cairns and Foster 1991; Foster and Cairns 1992; Hall 1992; Steele and Jinks-Robertson 1992). Given the general nature of the artifacts described above, it is at least conceivable that alternative explanations may exist for these cases as well. Rather than speculate on such alternatives, however, I next review several molecular mechanisms for directed mutation that have been proposed in conjunction with these cases. It is important to emphasize at the outset that none of these mechanisms has been validated empirically.

Proposed Mechanisms for Directed Mutation

Truly Directed Mutation: Specific Reverse Transcription

Only one mechanism for truly directed mutation has been proposed. Cairns et al. (1988) considered the possibility that "the cell could produce a highly variable set of mRNA molecules and then reverse-transcribe the one that made the best protein." If the cell could somehow monitor the protein products translated from these variable mRNAs and specify reverse transcription of the message for successful proteins, truly directed mutation would be the result. Such a highly specified reverse flow of information would require the presence of functional reverse transcriptase and a heretofore-unknown cellular component that monitors the success of proteins and chooses the appropriate mRNA for reverse transcription. There is little current support for this model. Reverse transcriptase has not been discovered in any of the *E. coli* K12 strains used to study directed mutation. In addition, extragenic suppressors of a *lacZ* amber allele arise in seemingly directed fashion during starvation on lactose, a phenomenon not predicted by the model (Foster and Cairns 1992).

Mechanisms that Do Not Invoke the Reverse Flow of Information

Mutagenic Transcription

Davis (1989) considered the possibility that transcription might be mutagenic such that the continuous pres-

ence of a selecting substrate that induces transcription (e.g., lactose) increases the mutation rate at the selected locus. Such a mechanism could produce the appearance of directed mutation, since beneficial mutations would arise at a higher rate only at the selected locus. However, the model also predicts that nonbeneficial (misdirected) mutations should arise at a higher rate at the selected locus, so transcriptional mutagenesis is not a truly directed mechanism.

Experimental evidence has not supported the transcriptional mutagenesis model. Although addition of the gratuitous inducer IPTG to growing populations of an inducible Lac^- strain does slightly increase the numbers of Lac^+ mutants observed, this effect is absent when IPTG is added to starving Lac^- populations, contrary to the prediction of the model (Davis 1989). In addition, the model does not explain why cells that constitutively transcribe the *lac* operon apparently accumulate mutations only in the presence of lactose (Cairns et al. 1988; Cairns and Foster 1991; Foster and Cairns 1992).

Slow Repair During Starvation

Stahl (1988) and Boe (1990) proposed that the methyl-directed mismatch repair system might act more slowly during starvation than during growth, allowing unrepaired sequence alterations on the transcribed strand to be fixed by chromosome replication if they enable cells to grow on the selective substrate. Mismatch repair-deficient strains do show elevated mutation rates under selective conditions (Boe 1990). However, the slow repair model predicts that uncorrected mismatch mutations should accumulate in a mismatch repair-deficient population that is starving regardless of whether selection is being applied. This is not the case: Lac^+ mutants apparently do not accumulate in Lac^- strains deficient in methyl-directed mismatch repair (or deficient in repair mediated by alkyltransferases) when lactose is not present (Foster and Cairns 1992). Nonetheless, deficient mismatch repair in starving cells is implicated by the spectrum of reversion mutations of a *lacZ* frameshift mutant recovered from cultures starving on minimal lactose medium (Rosenberg et al. 1994; Foster and Trimarchi 1994). These results are discussed further below.

RecA-Dependent DNA Replication

Cairns and Foster (1991) showed that RecA function is required for "adaptive" reversion of a *lacZ* frameshift mutant in *E. coli.* On the basis of this result, they proposed that amplification of mutant genes could explain the appearance of directed mutation in this system (Foster and Cairns 1992). Any duplicated copy that contained a mutation that conferred growth would allow the cell to leave stationary phase, after which the amplified region could be resolved by a RecA-dependent process. Stahl (1992) has noted that this model can only produce a high bias in favor of beneficial mutations if mutants that arise within a duplication or amplified array are subsequently and preferentially amplified further. Otherwise, any favorable mutation has a relatively high probability of being lost when the amplified region is resolved back to a single copy by random recombinational processes. The lack of any known process that could preferentially amplify beneficial mutant copies of a duplication casts serious doubt on this model.

Stahl (1992) has suggested a further model that is dependent on RecA function, which he calls the "toe in the water" model. This model invokes a form of DNA synthesis, called "stable DNA replication" (Demassey et al. 1984), which occurs in nondividing cells and is RecA dependent (Witkin and Kogoma 1984). Stahl has suggested that such replication might ordinarily halt at the D-loop stage during starvation, with subsequent degradation of the incipient strand. However, if a growth-enabling sequence change on the incipient strand could be transcribed, a full replication fork might form and the useful mutation would be transmitted to a daughter cell. At present there is no evidence in favor of this model other than the fact that stable DNA replication is RecA dependent.

The Hypermutable State Model

Hall (1990) proposed that a fraction of the cells in a starving population might enter a hypermutable state in which rampant DNA damage leads to growth if repair produces a favorable mutation but otherwise leads to cell death. This model could account for biased recovery of beneficial mutants if hypermutating cells bearing mutations that do not enable growth die eventually as a result of unrepaired damage. There is at present no evidence for the existence of subpopulations of hypermutating cells in any purported case of directed mutation. Harris et al. (1994), however, have recently presented evidence in one system that is consistent with a role for repair of lethal DNA damage in the generation of mutants during starvation. These authors have shown that, in addition to RecA, the RecBCD complex is required for "adaptive mutation" in the *lacZ* frameshift strain of *E. coli* studied by Cairns and Foster (Cairns and Foster 1991; Foster and Cairns 1992). RecBCD enzyme loads onto DNA at double-strand breaks and mediates repair via recombination between homologous sequences (Thaler and Stahl 1988). Such repair can be mutagenic, and Harris et al. (1994) suggest that "Double-strand break formation, allowing high-level recombination, could be the molecular basis of the hypermutable state that either kills or is stopped when an adaptive mutation . . . rescues the cell. Failure to make the adaptive mutation allows continued double-strand breakage, which kills."

Sequencing of revertants recovered during starvation of the *lacZ* frameshift strain on lactose minimal medium has revealed that the majority of these are the result of single-base deletions in small mononucleotide repeats (Rosenberg et al. 1994; Foster and Trimarchi 1994). In contrast, sequencing of revertants recovered from grow-

ing cultures indicates a broader spectrum of mutational events, including duplications, deletions, and insertions many nucleotides in length. Such a change in the relative frequencies of different mutations recovered suggests that certain mutational events are more frequent in starving *lacZ* frameshift cells than in growing cells. Rosenberg et al. (1994) and Foster and Trimarchi (1994) note that the mutations predominantly recovered from starving cells are similar to those associated with strand slippage during recombination, repair, or replication. Why such a mutational spectrum is only recovered from cultures starving on lactose minimal medium (why reversion of the *lacZ* frameshift appears directed), however, is still not understood. Rosenberg et al. (1994) have suggested a further elaboration of the hypermutable state model in which polymerase errors (possibly associated with strand slippage) occur during recombinational repair of lethal damage and are subsequently fixed as mutations as a consequence of decreased postsynthesis mismatch repair. Though this model is consistent with the sequence data and the requirement for the RecA and RecBCD functions, it must be considered speculative at present.

Mechanisms and the Causes of Apparently Directed Mutations

Among the models discussed above, only the "toe in the water" model of Stahl (1992) and the hypermutable state model of Hall (1990) are without obvious flaws. There is no specific experimental evidence lending plausibility to the "toe in the water" model, whereas the recent implication of RecBCD enzyme in the recovery of mutations from starving cultures of a *lacZ* frameshift mutant (Harris et al. 1994) is consistent with the hypermutable state model.

If the hypermutable state model (or a variant thereof) were to prove correct for the *lacZ* system, would this mean that an example of directed mutation had at last been demonstrated conclusively? Certainly not. The hypermutable state model relies on natural selection to mediate nonrandom recovery of mutants. Individual cells in this model reap the fitness consequences of random sequence alteration (unrepaired or erroneously repaired damage). For this reason, the hypermutable state is *not* a model for directed mutation. One might object that unrepaired lethal damage is not mutation since it is not inherited (an accepted definition of mutation is "a sudden heritable change in the genetic material": Lincoln et al. 1982), and that, therefore, the occurrence of mutations in the hypermutable state model would be nonrandom. Such an objection, however, is a semantic dodge that ignores the central importance of natural selection in the model; it is like saying that the occurrence of good hands in a poker game is nonrandom because some players drop out of the betting without showing their cards.

Conclusion

The radical suggestion by Cairns et al. (1988) that caused the original uproar over directed mutation was that "cells may have mechanisms for choosing which mutations will occur." Although this possibility (i.e., truly directed mutation) has been rejected by all sides, the debate over directed mutation somehow continues, mired in confusion over the causes and consequences of the origin of adaptive mutants. This confusion is reflected in the misleading use of such terms as "adaptive mutation" and "selection-induced mutation" to refer to nonrandom mutation. Despite a considerable body of evidence against directed mutation, its proponents persist in making unqualified references to the reality of "nonrandom mutations that occur as specific, direct responses to environmental challenges" (Hall 1993). No molecular mechanism for such nonrandom mutation, however, has been demonstrated. A currently plausible mechanism (the hypermutable state), in fact, is simply a form of natural selection between cells. Indeed, the only directed "mechanism" that has been conclusively shown to explain any case of apparently directed mutations *is* natural selection, as exemplified by the population-level processes reviewed by Lenski and Mittler (1993) and discussed at the outset of the present paper.

Hall (1988) has implied that the importance of mutation-rate variation has been neglected in evolutionary biology. This is hardly correct. The possibility that mutation rates may be adjusted in evolutionary response to the need for new adaptive mutants and other selective forces has been the subject of a considerable number of theoretical and empirical studies (Sturtevant 1937; Kimura 1960, 1967; Levins 1967; Leigh 1970, 1973; Eshel 1973; Cox and Gibson 1974; Painter 1975; Cox 1976; Gillespie 1981a,b; Chao and Cox 1983; Chao et al. 1983; Holsinger and Feldman 1983; Tröbner and Piechocki 1984a,b; Kondrashov 1988; Ishii et al. 1989; Drake 1991a). Recently, Moxon et al. (1994), building on these earlier studies of mutation-rate evolution, have reviewed several highly plausible cases of adaptive enhancement of mutation rates at specific loci in pathogenic bacteria. The possibility of such adaptive mutation is worthy of further research.

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