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LETTERS

"Adaptive Mutation": The Debate Goes On

In 1988, John Cairns *et al.* (*1*, p. 142) proposed that bacteria "may have mechanisms for choosing which mutations will occur" and thus challenged the tenet that mutations occur without regard to their effects on an organism's capacity to survive and reproduce. The directed mutation hypothesis requires that cells be able to sense their environment and, moreover, use this information to produce specific beneficial mutations. Several purported cases of directed mutation have now been contradicted by further experimental studies (*2*). The reports by J. Pablo Radicella *et al.* (21 Apr., p. 418) and Timothy Galitski and John R. Roth (21 Apr., p. 421), showing that plasmid transfer is intimately involved in the *lacZ* frameshift mutations in *Escherichia coli* strain FC40, also undermine earlier work by showing, once again, that the phenomenon in question is different from what was originally described (*3*).

PHOTO RESEARCHERS

Life after physics? Physicist Erwin Schrödinger, who introduced the term "directed mutations" in his 1944 book *What Is Life?* (Cambridge University Press, Cambridge, UK, pp. 35–36).

At the same time that the directed mutation hypothesis has accumulated what would seem to be several lethal hits, it has survived by acquiring a new name and, perhaps, a new identity. The new name is "adaptive mutation," but what does it mean? Biologists commonly refer to mutations that confer some selective advantage as being adaptive (or beneficial), in contrast to those that are maladaptive (or deleterious). Certainly there is nothing new there. However, in a recent Perspective, James A. Shapiro (21 Apr., p. 373) discusses a more interesting meaning of "adaptive mutation." According to Shapiro, an organism is like "a genetic engineer [with] an impressive toolbox full of sophisticated molecular devices for reorganizing DNA molecules." He contrasts this evolutionary analogy with that of the "blind watchmaker" (*4*). In particular, Shapiro suggests that conditions of "stress" or "selection" (*5*) may systematically promote mutations, a phenomenon that he believes is beneficial. Thus, the adaptiveness in "adaptive mutation" refers to the process of mutation, and not to each and every specific outcome.

However, adaptive mutation, like directed mutations, is a hypothesis, and there are plausible alternative hypotheses that must be considered. For example, an increased mutation rate in response to starvation (or any other such "stress") may be a pathological symptom of a cell that is losing control over its genetic integrity. A critical issue is whether a genotype whose mutation rate increases in response to starvation would tend to leave more descendants than one that did not have this response. It is not enough to show that there exists cellular machinery that encodes this response, as this is a necessary consequence of the phenomenon's existence and does not discriminate between "adaptive" and "pathological" explanations. Nor is it sufficient to show that certain beneficial mutations occur at higher rates under conditions of starvation than of resource abundance. Instead, one must attempt to evaluate rigorously the costs (increased deleterious mutations) as well as the benefits (occasional beneficial mutations) of a particular mutational response. One might presume that there is no cost to a starving cell of increasing its mutation rate, because that cell may die unless it acquires a mutation that allows it to use an available resource. But that presumption does not account for the possibility that the environment will subsequently become more permissive, in which case the better strategy might be to sit tight, rather than risk compromising an essential gene by mutation. So although it is plausible that organisms are rather like "genetic engineers," we caution against the fallacy of adaptationist reasoning, wherein each feature of every organism is taken to reflect the best of all possible worlds (*6*). Modern evolutionary theory is much more than adaptation by natural selection, as it also recognizes the possible roles of random genetic drift, pleiotropy, and various structural constraints in explaining the derivation of traits. It has been suggested, for example, that some "natural genetic engineering" may be an inadvertent consequence of the movement of parasitic genomes (for example, viruses and transposons), rather than an adaptation to promote new genetic combinations (*7*, *8*). We do not mean to reject

the hypothesis of "adaptive mutation," but rather to emphasize that alternative explanations must also be considered (9).

Finally, if some instances of "natural genetic engineering" are adaptations [as seems likely to us; see (*10*)], then how did such mechanisms evolve? Barring an appeal to vitalism, it seems that the "blind watchmaker" must be responsible! That is, natural selection must have differentially enriched or eliminated random mutations that affected various mutational processes, leaving present-day organisms with a "toolbox" that has allowed them to cope reasonably well with the environmental unpredictability that has characterized the history of life on Earth. Indeed, far from being news to evolutionary biologists, as Shapiro implies, the idea that the genetic systems of organisms may be tuned (by natural selection) to promote evolutionary success is an area of long-standing interest, as witnessed by a substantial literature on the evolution of sexual recombination and mutation rates (for example, *7*, *10*, *11*). What molecular biology provides is a mechanistic (proximate) understanding of these phenomena, which complements—but does not replace—an evolutionary (ultimate) understanding.

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1. J. Cairns, J. Overbaugh, S. Miller, *Nature* **335**, 142 (1988).
2. R. E. Lenski and J. E. Mittler, *Science* **259**, 188 (1993); P. D. Sniegowski, *J. Mol. Evol.* **40**, 94 (1995).
3. J. Cairns and P. L. Foster, *Genetics* **128**, 695 (1991).
4. R. Dawkins, *The Blind Watchmaker* (Norton, New York, 1986).
5. "Selection" refers to differential reproductive success among phenotypes or genotypes, and selection per se cannot cause mutations. In contrast, "stress" is a poorly defined biological concept. To avoid semantic problems, the hypothesis might be better phrased by saying that environments that cause low survival—and which may thereby have a large variance in reproductive success—are associated with high rates of mutation.
6. S. J. Gould and R. C. Lewontin, *Proc. R. Soc. Lond. B* **205**, 581 (1979).
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Response: Lenski and Sniegowski correctly interpret my distinction between "adaptive mutation" (useful mutations occurring more frequently when needed) and "directed mutation" (useful mutations occurring at specific genomic locations in response to particular selective conditions). The major difficulties with the directed mutation idea as originally proposed (*1*) were lack of clear experimental evidence and the establishment of a misleading dichotomy between extreme alternative control regimes for genetic change (blindness versus omniscience). Biological information processing does not have to be perfect to be a significant aspect of mutational response to selective challenge.

Molecular genetics has revolutionized our understanding of cellular mutational mechanisms. The new information alters some of our underlying assumptions. On the one hand, we now know that elaborate repair regimes take care of accidental genomic damage (for example, radiation and chemical insults, replication errors). Thus, these random events diminish as potential sources of evolutionary variation. On the other hand, it is now clear that cells contain multiple, sophisticated, natural genetic engineering systems (nucleases, ligases, topoisomerases, recombinases, transposons, retrotransposons, plasmids, viruses), and we increasingly appreciate these cellular biochemical activities as important mu-

tagenic agents (2). The versatile operations of these systems include insertion, deletion, inversion, fusion, amplification, dispersed and tandem reiteration, and other DNA rearrangements (3).

In contrast to Lenski and Sniegowski, I find it more reasonable to think of mutational events (which may involve many precise biochemical reactions) as resulting from the concerted action of dedicated cellular machines than as accidents or "pathologies." My argument is that these "high-tech" natural genetic engineering systems serve an adaptive function by generating the hereditary variability needed for short- and long-term survival. They provide the biochemical activities that account for evolutionary patterns of genome organization unanticipated by conventional theory: shuffling of sequences encoding protein domains, assembly of regulatory regions containing multiple transcription factor binding sites, duplication and dispersal of sequences among gene families, and amplification of repetitive DNA elements.

The emergence of bacterial antibiotic resistance did not follow neo-Darwinian predictions that bacteria would become resistant by accumulating mutations that alter cellular structures. Instead, bacteria acquired new genetic elements that encode special resistance mechanisms (4). The construction and spread of these resistance determinants depended on natural genetic engineering systems such as plasmids, transposons, and integrons (5). Functional utility is also apparent in developmental DNA rearrangements, such as those underlying B and T cell development (6) and ciliate macronuclear differentiation (7). These instances of efficient, coordinated genomic reorganizations are examples of the genetic engineering that is available to the evolutionary process.

Two questions about evolution concern the origins of genetic novelties and the role of informational feedback onto the genome. (i) What are the sources of evolutionary inventions? The best models for answering this question will come from studying how natural genetic engineering systems produce new DNA structures and alter genetic regulatory regimes. Studying creative DNA rearrangements is more relevant to evolution than analyzing point mutations that merely restore preexisting structures. (ii) How much informational feedback is there between the organism, its environment, and its genome? The signal transduction systems regulating natural genetic engineering are molecular neural networks that incorporate such feedback (8). Future research may discover just how "blind" or how "perceptive" these signal transduction systems are. We currently know little about evolution; that is why sequence database analysis continually produces surprises. So it is far too early in the game to speculate usefully about the origins of natural genetic engineering systems or to talk about any "ultimate understanding" of the evolutionary process.

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■

Adaptive Mutation and Sex

Three papers, two in the issue of 21 April (J. P. Radicella *et al.*, p. 418; T. Galitski and J. R. Roth, p. 421) and one elsewhere (*1*), show that adaptive mutation of a bacterial episome requires gene products that are also known to be involved in transfer of the episome during bacterial mating. Radicella *et al.* go further and imply, in their abstract and at other points in their report, that they have demonstrated an association between adaptive mutation and conjugal transfer of the episome, even though they have not actually tested that idea. They could have asked whether episomes that have been transferred are more likely to be mutant than those that have probably not been transferred; this would have been an easy experiment, but they apparently did not do it. They say that their experiments suggest, at the very least, "a requirement for the formation of mating aggregates" as the stimulus for mutation. If that were true, cells whose mating pili had been destroyed by prior exposure to the detergent SDS should be unable to undergo episomal mutations when diluted into top agar and put on selective plates. As it happens, this was the control for the experiment described in figure 4 of their report, and it showed that the episomes of SDS-treated cells seem to have a higher mutation rate than normal cells.

Seeking support for their ideas, Radicella *et al.* end their report by quoting Foster and Trimarchi (*1*) as having shown that mutation "requires that the *lac* allele be on the episome and is enhanced by the expression of conjugal functions." It would have been less misleading if they had included the next sentence, "However, actual conjugation is not required and, in our experiments, there is little evidence that episome transfer is mutagenic" (*1*, p. 5487).

The report by Galitski and Roth is more straightforward, and they offer a testable explanation why reversion of an episomal lac frameshift appears to be more frequent when it is adaptive. Unlike Radicella *et al.*, they do not imply that adaptive mutation requires conjugal transfer; indeed, Roth has written, "Our experiments do not suggest that the act of transfer is required for adaptive mutation" (*2*).

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1. P. L. Foster and J. M. Trimarchi, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5487 (1995).
2. J. R. Roth, personal communication.

Response: We have shown that about 10% of the post-plating revertants in the standard *lac* assay can be associated with the successful transfer of the episome carrying the revertible allele from the indicator cells to the scavenger cells (figure 1 in our report). Our experiments (note 15 in our report) and those of Foster and Trimarchi (*1*) show that only between 0.1 to 1% of the episomes are transferred to the scavenger under those conditions. We therefore suggest that the mutations can be associated with transfer. As to the question of whether episomes that have undergone transfer display an elevated incidence of mutation, we have not made this claim; but Kunz and Glickman (*2*) have reported substantial episomal marker mutability associated with conjugal transfer. Attention has been drawn to this observation by Taddei *et al.* in an accompanying letter.

As to whether or not cells grown in the presence of SDS should be able to undergo episomal mutations when put on selective plates in the absence SDS, we suggest that the leakiness of the *lacI33* allele is sufficient to allow reassembly of the pili required for the generation of conjugational signals during selection. The two- to threefold greater yield of revertants seen in the control experiment (our figure 4) on which Cairns comments is within the range of experimental variability evident among independent cultures and in reported studies (*3*, *4*).

We have not argued that conjugal transfer must always be successful. We acknowledge that, even in the presence of an excess of scavenger cells, the majority of the reversion events occur in the indicator cells. The importance of conjugation need not depend on the successful completion of conjugal transfer. For example, the transferred DNA could fail to replicate in the recipient cells (most likely scavenger) and therefore be lost. The issue may simply be how many times, during prolonged selection, the F′ plasmid of the indicator bacteria has experienced a replication as a consequence of the initiation of the conjugation process. The success or failure of the transfer need not matter.

The suggestion by Cairns that we intended to mislead in not quoting a conclusion we do not find compelling does not do him credit.

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■

Mutation Rate of the F Episome

The mutation rate per replication of all DNA-based genomes (including viruses like M13 and lambda, bacteria, and yeast) appears to be constant (on the order of one per 300 genomes replicated—Drake's rule) (*1*). This constant likely reflects an optimal or minimal rate of mutation. There is one noticeable exception to this rule, the F episome. Although the F episome is not generally considered itself a microorganism,