10, 137 (1963)]. Analysis of variance was used to compare neuronal responses for each trial type within the cue and delay conditions. The source of significant variance was then determined by the Tukey HSD test. All delay-related and stimulus-specific neuronal responses were based on significant comparisons. Care of the monkeys was in accordance with the Yale University Animal Care Committee.

8. On PDR trials, neuron 787 responded during the delay period (26 spikes per second) after a pattern that required a rightward saccade and was significantly less responsive (12.5 spikes per second) after the other pattern, and after spatial cues (11 and 10 spikes per second) on SDR trials. Spontaneous activity was 8.4 spikes per second.
9. The delay period activity of neuron 627 on SDR trials was 13.5 spikes per second after spatial cues requiring saccades 13° to the right, significantly greater than the delay activity (2.9 spikes per second) for leftward-going saccades, and the delay activity (4.2 and 0.9 spikes per second) on PDR trials. Spontaneous activity was 1.9 spikes per second.
10. The picture fixation task required the monkey to fixate while stimuli (subtending 10°) were presented in foveal vision for 1 s, and juice was delivered 0.5 s after stimulus offset; directional saccades were not required. This task utilized a library of 280 stimuli, grouped into 40 sets of seven images (five objects, one color field, and one face). The stimuli were digitized photographs of laboratory objects; each color field was of uniform hue, generated by mixing red-green-blue values with graphics software.
11. Neuron 787: a total of 30 objects was presented (294 trials), of which 22 elicited a response (range, 37.6 to 14.3 spikes per second, mean, 24 spikes per second; spontaneous activity, 8.4 spikes per second); the other 8 objects failed to elicit any change in firing rate. The neuron (Figs. 1B and 2A) was unresponsive on SDR trials (80 trials) when the monkey had to remember and respond to the locations of 8 spatial cues; the average response to these 8 cues was 8.1 spikes per second.
12. D. I. Perrett, E. T. Rolls, A. W. Caan, *Exp. Brain Res.* **47**, 329 (1982).
13. The responses of neuron 566 to faces ranged from 99 to 30.6 (mean, 64) spikes per second for 12 face stimuli presented, significantly greater than the responses to 14 objects ranging from 23 to 8.2 (mean, 16.4) spikes per second; data were obtained on 240 trials of the picture task. The neuron was unresponsive (mean, 11.8 spikes per second) in the eight-location SDR task (80 trials). Spontaneous activity was 16.2 spikes per second.
14. Neuron 532 fired during the delay period over which the monkey was required to remember one particular face (11.8 spikes per second), a response significantly greater than the responses to another face (3.9 spikes per second) and to the two patterns (6.1 and 5.9 spikes per second). Spontaneous activity was 5.3 spikes per second.
15. C. G. Gross, C. E. Rocha-Miranda, D. B. Bender, *J. Neurophysiol.* **35**, 96 (1972); R. Desimone, T. D. Albright, C. G. Gross, C. J. Bruce, *J. Neurosci.* **4**, 2051 (1984).
16. E. K. Miller, L. Li, R. Desimone, *Science* **254**, 1377 (1991); I. P. Riches, F. A. W. Wilson, M. W. Brown, *J. Neurosci.* **11**, 1763 (1991).
17. K. Kawamura and J. Naito, *Neurosci. Res.* **1**, 89 (1984); H. Barbas, *J. Comp. Neurol.* **276**, 313 (1988).
18. K. A. Macko *et al.*, *Science* **218**, 394 (1982).
19. Stimulus-selective neuronal activity in the inferior convexity did not depend on directional eye movements, but we have recorded dorsolateral prefrontal neurons with conditional responses to foveal stimuli when the stimuli required a specific movement in the PDR task.
20. A. Incisa della Rochetta, *Cortex* **22**, 189 (1986); R. A. McCarthy and E. K. Warrington, *Cognitive Neuropsychology* (Academic Press, London, 1990).
21. M. Corbetta *et al.*, *J. Neurosci.* **11**, 2383 (1991); D. A. Weinberger, K. F. Berman, R. F. Zec, *Arch. Gen. Psychiatr.* **43**, 114 (1986).
22. This research was supported by National Institutes of Mental Health grants MH44866 and MH38546 and by McDonnell Foundation grant JSMF 91-47.

18 December 1992; accepted 1 April 1993

TECHNICAL COMMENTS

Unusual Mutational Mechanisms and Evolution

R. E. Lenski and J. E. Mittler argue that studies purporting to demonstrate directed mutation lack certain controls and do not account adequately for population dynamics (*1*). In addition, none of the novel, even unorthodox, mechanisms invoked to explain the directed mutation hypothesis is supported by secure evidence, and some of the ideas have proved untenable (*1–5*). In concluding that no evidence has been presented to deny the classical tenet that mutation and selection are independent, Lenski and Mittler point out that mutation rates may vary both between and within genomes, and they raise the possibility that variable mutation rates may be an evolved response that specifically promotes increased genetic variation under stress. We lean favorably toward this suggestion and wish to expand the proposition with reference to documented genetic mechanisms.

Central to the directed mutation debate is a means of explaining the increased frequency of altered phenotypes, which those who support the hypothesis have said arises "specifically when (and even because) it is advantageous" (*1*). We suggest that an increase in the frequency of altered phenotypes could occur as the result of an increase in the frequency of gene expression mediated by two classical mechanisms, namely, alterations in DNA sequence and alterations in DNA topology. Mechanisms facilitating alterations in the frequency of gene expression include reiterative oligonucleotide sequence motifs, which introduce frameshifts and affect translation (*6*); homopolymeric tracts, which, because of the likelihood of insertions or deletions occurring within regions of reiterated bases, affect transcription (*7*); and differential inhibition of site-specific methylation, which induces extrinsic alterations in DNA conformation (*8*). These are but a few examples, possibly the tip of the iceberg, of documented mechanisms with potential for mediating high-frequency changes in DNA sequence, or DNA confirmation. We wish to emphasize that, in addition to being compatible with neo-Darwinian theory, these mechanisms contribute an intrinsically stochastic component to the regulation of gene expression, the potential outcome of which is polymorphism, that is, population heterogeneity. Far from being directed, such phenotypic variations arise from mechanisms that are blind.

In an in-press article we have placed considerable emphasis on the potential importance of polymorphism within bacterial populations generated through genetically based stochastic mechanisms (*9*). We also outlined evidence suggesting a role for stress in regulation of the frequency of altered gene expression.

Mechanisms capable of generating random variation might provide a satisfying solution to the problem of responding to unpredictable environmental change. It is likely that evolution has favored a balance between biological memory (as stored within a sequence of nucleotides) and probabilistic mechanisms ("contingency behavior") that enables an optimal response to situations that cannot be anticipated.

Paul Rainey
Molecular Microbial Ecology,
Institute of Virology and
Environmental Microbiology,
Mansfield Road,
Oxford OX1 3SR, United Kingdom
Richard Moxon
Molecular Infectious Diseases Group,
Institute of Molecular Medicine,
John Radcliffe Hospital,
Oxford OX3 9DU, United Kingdom

REFERENCES

1. R. E. Lenski and J. E. Mittler, *Science* **259**, 188 (1993).
2. J. Cairns, J. Overbaugh, S. Miller, *Nature* **335**, 142 (1988).
3. F. W. Stahl, *ibid.*, p. 112.
4. B. D. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5005 (1989).
5. B. G. Hall, *Genetics* **126**, 5 (1990).
6. J. N. Weiser, J. M. Love, E. R. Moxon, *Cell* **59**, 657 (1989).
7. D. Yogev, R. Rosengarten, R. Watson-McKown, K. S. Wise, *EMBO J.* **10**, 4069 (1991).
8. B. A. Braaten, L. B. Blyn, B. Skinner, D. A. Low, *J. Bacteriol.* **173**, 1789 (1991).
9. P. B. Rainey, E. R. Moxon, I. P. Thompson, *Adv. Microb. Ecol.*, in press.

5 March 1993; accepted 17 May 1993

The article by Lenski and Mittler (*1*) prompted us to reevaluate our data about the conjugative transposable element Tn*916* (*2*). Our data suggest that control of

Tn916 excision may be influenced by external factors, including exposure to aminoglycoside antibiotics. Differential gene regulation during growth, or a switch to stress or heat shock promoters in response to antibiotic pressure, could effectively derepress transposon excision and therefore increase apparent mutation rates. This would offer no selective advantage to the host, but might to the transposable element by providing the transposon with an extremely simple method for sensing optimal timing of excision, which is the initial and rate-limiting step in transposition; such a sensor would enhance the ability of the element to survive a selection event during which its former host is killed by increasing its ability to excise and subsequently integrate into a new host having greater fitness. The effect of this would be to promote the survival of this "selfish" element, even over that of the host. By extension, in bacterial genomes, one could hypothesize that pairs of transposons might coordinately excise under selective conditions (including, for example, nutrient starvation) and as a result delete or possibly rearrange large portions of the bacterial chromosome.

David A. Watson
Department of Clinical Microbiology, Academisch Ziekenhuis Utrecht, Heidelberglaan, 3508 GA Utrecht, The Netherlands

REFERENCES

1. R. E. Lenski and J. E. Mittler, *Science* **259**, 188 (1993).
2. D. A. Watson, D. M. Musher, R. J. Hamill, in *Abstracts of the 92nd General Meeting of the American Society for Microbiology* (American Society for Microbiology, New Orleans, LA, 1992), no. H-4, p. 183.

3 February 1993; accepted 17 May 1993

Lenski and Mittler (*1*) defend Darwinism in a valuable, close review of the available evidence on directed mutation in one class of experiments. Their main conclusion is that no directed mutation has been convincingly demonstrated. Lenski and Mittler's position is supported by an example from a different field which convincingly demonstrates directed mutation, but which still poses no threat to Darwinism.

The well-described phenomenon of repeat induced point mutation (RIP) (*2*) is a directed mutational process. In *Neurospora crassa* and *Ascobolus immersus*, DNA duplications that enter the sexual cycle are usually extensively methylated, which leads to their immediate functional inactivation. In *N. crassa*, methylation at cytosines is accompanied by a high rate of G·C → A·T mutation, so-called ripping (*2*). The duplicate elements that trigger RIP suffer equivalent but nonidentical damage (*2*). A related phenomenon has been implicated in other eukaryotes, most particularly mammals (*3*), and may account for a high percentage of base pair substitutions within these lineages (*3*).

The function of ripping is not fully understood. Ripping may help protect the genome by degrading parasitic mobile genetic elements as soon as they duplicate (*2*). The genome of *N. crassa* has a dearth of duplications. Ripping may reduce the rate of abnormal meiotic pairing and of translocations (*2*) by reducing nonhomologous recombination. Linked sequences that have been ripped are poor substrates for intrachromosomal recombination (*4*). We assume that ripping is advantageous for one of these reasons, or for a similar reason.

Mutations are directed if their rate of occurrence depends on the environment, and if that dependence is in an adaptive direction. Two environments may be distinguished for a genome: (i) the presence of duplications and (ii) the absence of duplications. Ripping occurs only in environment (i) and is adaptive there, but it does not occur in (ii), in which it would be maladaptive. Ripping occurs at a high frequency with perfect homology but declines as homology declines, ceasing at about 14% divergence (*5*). Ripping does not affect duplicate copies of the three large ribosomal RNA (rRNA) molecules in their normal position within the genome (*2*), and in this gene specificity, too, ripping is directed.

Ripping contrasts with the systems reviewed by Lenski and Mittler (*1*): The environmental trigger in the case of ripping (that is, the presence of duplications) is internal to the cell. Also, the effect of ripping is usually to degrade the mutated gene. Nevertheless, the cell's mutational response is specific to a particular cue, specific to particular genes, and on the average advantageous. It is a directed mutational process, even if it is one that degrades information rather than creates it.

Evidence suggests that no novel processes are involved in the mechanism of ripping. Recombination and RIP are most probably mechanistically related (*2*). If three copies of a gene are present, one may be left unaltered (*6*); if two copies are present, either both or neither will be altered. It is probable that RIP mutation is associated with replication (*2*) and most probably with the dismantling of inappropriately paired sequences (*2*). During this dismantling, a specific RIP machinery may be functional (*2*). Thus, in contrast with unorthodox explanations of directed mutation (see *1*, *7* for review), ripping is probably an unexceptional molecular genetic process.

Laurence D. Hurst
Department of Zoology, South Parks Road, Oxford OX1 3PS, United Kingdom

Alan Grafen
Department of Plant Sciences, South Parks Road, Oxford OX1 3RA, United Kingdom

REFERENCES

1. R. E. Lenski and J. E. Mittler, *Science* **259**, 188 (1993).
2. E. U. Selker, *Annu. Rev. Genet.* **24**, 579 (1990).
3. M. C. Kricker, J. W. Drake, M. Radman, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1075 (1992).
4. E. B. Cambareri, M. J. Singer, E. U. Selker, *Genetics* **127**, 699 (1991).
5. E. J. Foss, P. W. Garrett, J. A. Kinsey, E. U. Selker, *ibid.*, p. 711.
6. J. R. S. Fincham, I. F. Connerton, E. Notarianni, K. Harrington, *Curr. Genet.* **15**, 327 (1989).
7. J. W. Drake, *Annu. Rev. Genet.* **25**, 125 (1991).

11 March 1993; accepted 17 May 1993

Response: Rainey and Moxon discuss molecular mechanisms with the potential to cause high rates of mutation in certain genes. They suggest that these mechanisms have evolved as a means of generating variation, which would allow more rapid evolutionary responses in changing environments, even though the resulting mutations are apparently random with respect to their selective value. We agree that this is an interesting hypothesis and one that is compatible with neo-Darwinian theory. A major area of ongoing research in evolutionary biology is concerned with the adaptive significance of those aspects of physiology, biochemistry, and reproductive biology that affect rates of mutation and recombination (*1*). There are costs as well as benefits to increasing the production of genetic variation: Although it may accelerate the rate of adaptive evolution in a changing environment, it may also destroy genotypes that are already well adapted.

A possible evolutionary advantage of the nonstandard mutational mechanisms mentioned by Rainey and Moxon is that they increase variability only at specific loci, in contrast to more familiar mechanisms that affect mutation rates throughout the genome (*2*). Such nonstandard mechanisms may concentrate mutations in loci that interact unpredictably with the environment, and without increasing the load of deleterious mutations elsewhere in the genome. For example, when pathogenic bacteria confront sudden and unpredictable environmental changes as a result of host immune responses and interhost variability, successful infection usually depends on specific characteristics of the bacterial cell envelope. These traits are often encoded by genes subject to nonstandard mutational mechanisms that ensure highly labile phenotypes (*3*).

Rainey and Moxon also suggest that stress may increase the rate of these nonstandard mutations. We recognize this

possibility but emphasize that, in evaluating the possible effect of stress on mutation rates, one must account for any differences in population dynamics in stressful and nonstressful environments. One of the important lessons of the directed mutation controversy is just how difficult it is to rigorously quantify mutation rates, which cannot be measured directly but must be inferred indirectly from the population dynamics of mutants and their progenitors (*4*).

Watson hypothesizes that excision allows the conjugative Tn*916* to escape from an ill-fated genome. We agree that it is important to consider the consequences of transposon-induced mutations to the fitness of a transposon as well as its host, and we have similarly suggested that *Mu* excisions may arise as that transmissible element attempts to leave a starving host (*5*).

Hurst and Grafen describe the interesting phenomenon of ripping in fungi. Ripping appears to have evolved to eliminate parasitic genetic elements, such as transposons, that spread by making copies of themselves and to prevent nonhomologous pairing during meiosis (*6*). The question raised by Hurst and Grafen is whether ripping qualifies as directed mutation. According to our definition of directed mutation (*4*, p. 188), "comparable increases in rate do not occur . . . for the same mutation in similar environments where it is not advantageous." With respect to ripping, Hurst and Grafen define similar environments on the basis of whether or not a gene duplication has occurred, and they conclude that ripping mutations occur only when they are advantageous. However, an occasional gene duplication that had fortuitously beneficial effects would also presumably be subject to ripping (in which case the ripping mutations would be misdirected). Thus, we regard ripping as a mutational process that is advantageous on average, rather than a process that occurs at a higher rate specifically when the mutation is advantageous.

Our difference with Hurst and Grafen, therefore, seems to be a matter of semantics. Our restrictive definition for directed mutation was intended to (i) emphasize the need for proper controls in experimental tests of the directed mutation hypothesis; (ii) distinguish that hypothesis from others concerning possible advantages of elevated rates of random mutation in certain genes or under certain circumstances; and (iii) highlight the issue of whether an organism can somehow test the value of a novel phenotype before introducing the corresponding change into its genome.

Ripping is similar in certain respects to other processes that repair damaged DNA and protect against invasion by foreign DNA. For example, methyl-directed repair preferentially resolves DNA mismatches in favor of the unchanged allele (*7*), while restriction-modification immunity tends to exclude the incorporation of foreign DNA into the genome (*8*). Like ripping, these processes appear to be adaptations to restore the status quo to a genome, rather than adaptations to generate variation that might allow an organism to track a changing environment. Thus, they provide an interesting contrast to the mechanisms discussed by Rainey and Moxon. None of these mechanisms, however, involves the reverse flow of information from phenotype to genotype, which might allow an organism to test the value of a novel phenotype before introducing the corresponding change into its genome, as Cairns *et al.* (*9*) so provocatively proposed.

Richard E. Lenski
Center for Microbial Ecology,
Michigan State University,
East Lansing, MI 48824
John E. Mittler
Department of Biology,
Emory University,
Atlanta, GA 30322

REFERENCES AND NOTES

1. E. G. Leigh, Jr., *Genetics* (suppl.) **73**, 1 (1973); J. Jaenike, *Evol. Theor.* **3**, 191 (1978); L. Chao and E. C. Cox, *Evolution* **37**, 125 (1983); R. E. Michod and B. R. Levin, Eds., *The Evolution of Sex* (Sinauer, Sunderland, MA, 1988); G. Bell, *Sex and Death in Protozoa* (Cambridge Univ. Press, Cambridge, United Kingdom, 1988); W. D. Hamilton, R. Axelrod, R. Tanese, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3566 (1990); M. Nowak, *Nature* **347**, 522 (1990); L. Chao, T. R. Tran, C. Matthews, *Evolution* **46**, 289 (1992). Darwin remarked that "Some authors believe it to be as much a function of the reproductive system to produce individual differences, or very slight deviations of structure, as to make the child like its parents" [C. Darwin (1859), *The Origin of Species by Means of Natural Selection* (Penguin, New York, 1976), p. 173].
2. E. C. Cox, *Annu. Rev. Genet.* **10**, 135 (1976); J. W. Drake, *ibid.* **25**, 125 (1991).
3. P. B. Rainey, E. R. Moxon, I. P. Thompson, *Adv. Microb. Ecol.*, in press.
4. R. E. Lenski and J. E Mittler, *Science* **259**, 188 (1993).
5. J. E. Mittler and R. E. Lenski, *Nature* **344**, 173 (1990); *ibid.* **345**, 213 (1990).
6. E. U. Selker, *Annu. Rev. Genet.* **24**, 579 (1990).
7. S. R. Kushner, in Escherichia coli *and* Salmonella typhimurium, F. C. Neidhardt, Ed. (American Society for Microbiology, Washington, DC, 1987), pp. 1044–1053.
8. S. E. Luria, *Cold Spring Harb. Symp. Quant. Biol.* **18**, 237 (1953); W. Arber and S. Linn, *Annu. Rev. Biochem.* **38**, 467 (1969).
9. J. Cairns, J. Overbaugh, S. Miller, *Nature* **335**, 142 (1988).

24 March 1993; accepted 17 May 1993

Evidence of Genetic Heterogeneity in the Long QT Syndrome

The long QT syndrome (LQT) is a familial predisposition to sudden death from cardiac arrhythmias (*1*, *2*). M. Keating *et al.* performed linkage analysis in a large Utah family and found that the disease was closely linked to the Harvey *ras*-1 (H-*ras*-1) locus on chromosome 11 (*3*). With the use of the probe pTBB-2 at the H-*ras*-1 oncogene, a logarithm of the likelihood ratio for linkage (lod score) of +16.44 was obtained by Keating *et al.* In a subsequent study (*4*), tight linkage of LQT to the H-*ras*-1 locus was found in six other small LQT families. The combined lod score from these two studies was +21.65 at a recombination fraction of 0. This tight linkage suggests that mutations at the H-*ras*-1 locus or at a closely linked locus resulted in LQT in the families studied.

In view of the clinical heterogeneity and possible genetic heterogeneity in this syndrome, we analyzed a large Jewish family with a history of LQT. This family, whose origin is the island of Jerba near Tunis and whose members reside in Israel, is probably the largest family with LQT outside the United States. It comprises 131 individuals, of whom 28 have been affected (Fig. 1).

Clinical and electrocardiographic data collected over 7 years were available for 92 family members and blood samples for

Table 1. LQT status by age and gender. The QT interval was measured in standard lead II and corrected for heart rate with the use of the Bazett's formula (*8*). Multiple electrocardiograms recorded over several years were available for most living family members. To account for the possible variability over time in QT_c per individual, QT_c phenotypic cutoffs were applied to the mean QT_c of all measured QT_c intervals per individual.

Status	Demographic subset [mean QT_c (in seconds)]		
	Children	Adult males	Adult females
Affected	>0.46	>0.45	>0.47
Unaffected	<0.44	<0.43	<0.45
Equivocal	0.44 to 0.46	0.43 to 0.45	0.45 to 0.47