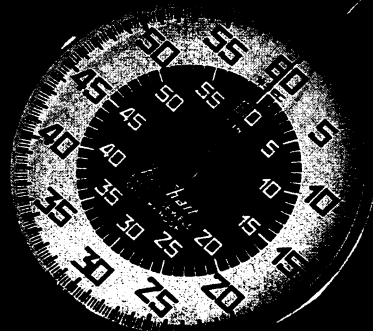




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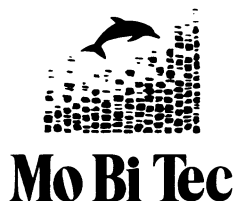
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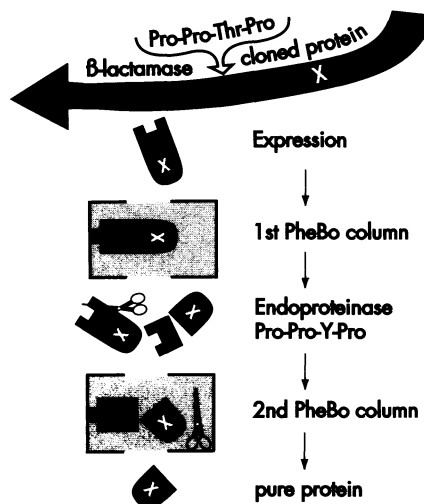
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www.ncbi.nlm.nih.gov/dbEST/index.html.

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Adaptive Mutation

The report "Recombination in adaptive mutation" by Reuben S. Harris *et al.* (8 Apr., p. 258) demonstrates the role of biochemical machinery for homologous recombination in adaptive reversion of a *lacZ* gene frameshift mutation. The accompanying Perspective by David S. Thaler "The evolution of genetic intelligence" (p. 224) describes the flow of information between the environment, the cellular activities that can reorganize DNA molecules, and the genome.

Our knowledge of the cellular basis of mutation was revolutionized by Barbara McClintock's discovery of transposable elements in maize and her demonstration of their ability to generate chromosome rearrangements and new alleles at individual genetic loci (1). An early example of adaptive mutation in bacteria involved the ability of a transposable element, phage Mu, to form *araB-lacZ* hybrid protein coding sequences with kinetics that were incompatible with the Luria-Delbruck concept of stochastic mutation (2). The importance of transposable elements has been relatively neglected in the debate about adaptive mutation because point mutations have been considered to be more relevant to evolutionary change. Examination of sequence databases, however, has shown that cut-and-splice processes must have been a part of the evolution of loci encoding multidomain proteins and of 5' regulatory regions, which are mosaic composites of many repetitive elements that specify the binding of transcription factors. As transposable elements encode precisely the kind of cleavage and ligation activities that can mediate the required DNA rearrangements, and as their movements frequently create new regulatory configurations, their functions could serve as models for certain evolutionary processes.

The basic similarity between the role of transposable elements in mediating DNA

rearrangements and the results of the report by Harris *et al.* is that both classes of physiologically sensitive genetic changes involve the action of biochemical complexes whose functions are to restructure DNA sequences. These natural genetic engineering systems are subject to cellular control regimes in the same way as are any other set of biochemical activities (3), thus, many important mechanisms of genetic change properly belong in the regulatory context of cell biology rather than in the statistical context of physics and chemistry.

James A. Shapiro

Department of Biochemistry and
Molecular Biology,
University of Chicago,
920 East 58 Street,
Chicago, IL 60637, USA

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Thaler points out that an important step in the understanding of the phenomenon known as "adaptive mutation" is the realization that, during selection, mutations occur by a different mechanism than they do in the absence of selection. Several years ago, John Cairns and I discovered that adaptive reversion of a frameshift allele of the *lacZ* gene requires a functional *recA* gene, whereas reversion occurring during normal growth does not (1). Thaler does not mention that our experiments also showed that adaptive mutation does not require activities known to be involved in SOS (the DNA damage repair response), a result that eliminated the known functions of *RecA* other than those involved in recombination (1, 2).

The report by Harris *et al.* specifically implicates the *RecBC* pathway of recombination by demonstrating an additional requirement for *RecBC*. Because *RecBC* is known to interact with duplex DNA ends, these results appear to exclude the hypothesis that adaptive reversion of this *lac* allele occurs during DNA synthesis primed by RNA:DNA hybrids (3). But the results do not distinguish between the alternative hypotheses that the mutations are created by *RecA*-dependent recombination per se, or that the mutations occur during DNA synthesis primed by *RecA*-dependent homologous pairing. Indeed, the priming of DNA synthesis by D-loops is a stress-induced response in *Escherichia coli* (4).

It should also be noted that the requirement for *RecA* function is not universal. Harris *et al.* used the same bacterial strain that we used (1). Although Jayaraman (5) also reported a requirement for *RecA* for adaptive mutation of a different mutational

target, there are examples of mutations that occur under selective conditions in the absence of *RecA*, including reversion of other *lac*⁻ alleles and of amino acid auxotrophies (2, 6). But, in order for mutations to arise in nondividing cells, there may be a universal requirement for special ways to initiate DNA synthesis, of which the *RecA*-dependent mechanism may be just one example.

Patricia L. Foster

Boston University School of Public Health,
Boston University School of Medicine,
80 East Concord Street,
Boston, MA 02118, USA

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Response: In her 1983 Nobel lecture (1), Barbara McClintock said,

A goal for the future would be to determine the extent of knowledge the cell has of itself and how it utilizes this knowledge in a "thoughtful" manner when challenged.

Shapiro (2), in the discussion of his 1984 paper, pointed out that the experiments by S. E. Luria and M. Delbruck (3), H. Newcomb (4), and J. Lederberg and E. M. Lederberg (5) involved immediately lethal selection and could only detect mutants that had originated in the absence of selection. He ended this important paper with the statement,

Indeed, now that we know about mobile genetic elements, inducible mutator systems and multiple biochemical activities that reorganize DNA molecules, the most pertinent questions in studies of hereditary change must be questions of control and regulation.

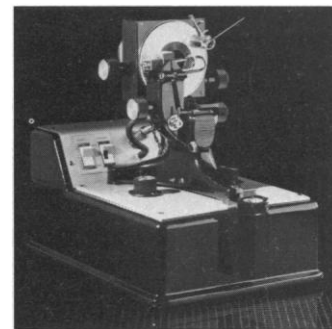
F. W. Stahl (6) has pointed out that the distinction between lethal and nonlethal selections was carefully made by Delbruck in 1946 in a comment on selection for carbon source utilization. Mutation under nonlethal selection and its deviation from Luria-Delbruck kinetics was the subject of extensive experimental work by Francis Ryan in the late 1950s and has been recently reviewed (7).

The special contribution of John Cairns and his colleagues (8) is that the distinction between generalized stress responses and

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"thoughtful" reactions of the organism was for the first time explicitly incorporated into experimental protocols. The adequacy of the methods used has been the subject of much critique and refinement; but the field of adaptive mutation, while not getting calmer, has—on average—been reaching higher intellectual levels ever since. F. W. Stahl (6) pointed out that if Ryan had included nonselected genes in his experiments, adaptive mutation as discussed at present would have been discovered before 1960. Shapiro's 1984 paper does not include generalized stresses; when the experimental design was extended to include tests inherent in the work of Cairns *et al.* (8), the results indicated a more generalized stress response (9).

Transposon involvement in adaptive mutation (that is, with controls for nonselected genes) has been the subject of work by Hall (10). Shapiro's study of the induction of phage Mu is profound, yet—as pointed out by R. E. Lenski and J. E. Mittler—control genes and regimes are essential when one considers specificity (11). Point mutations, recombination, and rearrangement are all the stuff of evolution.

The work of Foster and Cairns (12) indirectly implicated recombination by the process of elimination, and "As several players have noted, the next step will be to sort out what genes of DNA metabolism are required for directed mutation" (13). DNA synthesis of a special sort is central to many models of mutagenesis (14). Nontargeted mutagenesis resulting from the SOS system response to DNA damage (15), with its mechanism of error-prone synthesis and implications for "inducible evolution" (16), is a good basis for extending the concept to adaptive evolution by means of specification in genome space as well as the original meaning of time.

David Thaler
Rockefeller University,
1230 York Avenue,
New York, NY 10021-6399, USA

References

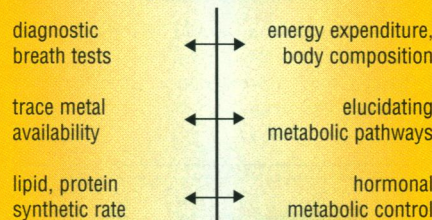
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Opinion

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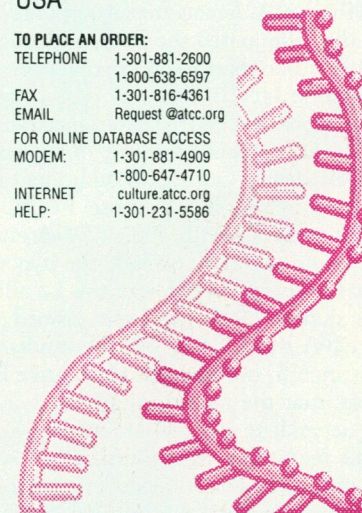
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