ELSEVIER

Mutation Research 473 (2001) 255–257

Fundamental and Molecular Mechanisms of Mutagenesis

www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Current Issues in Mutagenesis and Carcinogenesis No. 99

Temporal control of environmentally responsive hypermutation involving cryptic genes

Michael M. Lieber*

1619 Hopkins St. #101, Berkeley, CA 94707, USA

Accepted 29 August 2000

Some bacteria are late in their utilization of various sugars. Late mutations, enabling such utilization, occur in hitherto hidden, not easily accessible genetic regions [1]. Such genetic regions, with no apparent history of involvement, have been referred to as cryptic genes. Relevantly, a gene enabling growth on lactose medium is not present in a strain of *Escherichia coli*. Yet, very frequent, late occurring, responsive mutations, which enable such growth, nevertheless occur in another, previously hidden genetic region [1].

In a pertinent study, one isolated an adaptively responsive mutator system in *E. coli*. This system is based upon chromosomally incorporated transposons originating from a mutant P1 plasmid present in the bacteria [2–4]. In such a transposon-based mutator system, the bacteria lack chromosomal regions containing genes involved in arginine and proline synthesis. The bacteria are Arg^- Pro^-. In such a bacterial mutator system, many frequent, concurrent mutations to Arg^+ Pro^+ nevertheless occur while the bacteria are on a growth medium lacking both arginine and proline [2–4]. This indicates mutation in an unknown number of cryptic genes controlling arginine and proline synthesis. This is in effect a non-local suppression of arginine and proline auxotrophy.

These frequent, concurrent mutations occur on a selective medium mostly during later incubation. It is a period between 3 and 7 days after the bacteria have been plated on the medium [3]. This is very similar to other adaptively responsive mutation systems in which various, adaptively responsive mutations generally occur during a broad period of later incubation [1,5–8]. The frequency of concurrent mutations to Arg^+ Pro^+ was recently compared to the predicted mutation frequency to both Arg^+ and Pro^+ in non-mutator bacterial strains under non-selective conditions. Under selective conditions, the concurrent mutation frequency in the mutator system was at least 10^{11}-fold greater than the predicted frequency. This clearly indicates a non-local, hypermutagenic response to environmental stress on part of a mutator system's cryptic features.

Furthermore, bacteria containing this transposon-based hypermutator system were transduced with P1 virions at a multiplicity of infection of 0.1. After transduction, the same, ultra-high, concurrent mutation to Arg^+ Pro^+ occurred between 0 and 2 days incubation under selective conditions, rather than within the last 4 days [3]. This much earlier, concurrent mutation frequency, occurring within a much shorter time-span, was also 10^{11}-fold greater than the predicted frequency.

In many similar experiments, there was repeated confirmation of this temporal effect operating in hy-

* Fax: +1-925-254-7800.
E-mail address: michaellieber@juno.com (M.M. Lieber).

PII: S0027-5107(00)00127-5

permutation due to additional P1 elements [3]. Furthermore, recent re-analysis of collective data [3,4] indicated the following: distribution of Arg^+ and Pro^+ mutants, generated by the mutator strain within 2 days after transduction, conformed to a Poisson distribution. This provided further evidence that stressful culture conditions also played a role in the earlier occurrence of the hypermutation implicating cryptic regions. This earlier occurrence of exceedingly high mutation was only confined to the transductants of those bacteria that were mutator strains.

Transduction of additional genetic elements into the genome of a bacterial mutator system, it can be concluded, has enabled adaptively responsive hypermutation to occur in a much earlier period, and within a much shorter time. A period differential and shortened time-frame was repeatedly indicated. This has important significance. Namely, the addition of further genetic elements of a certain type to the genome in a mutator system, based on transposons already present in the genome, can greatly compress in time the occurrence of a very high degree of adaptively responsive mutagenesis. More generally, this implies an environmentally responsive, temporal control of adaptive hypermutation involving cryptic genetic regions of unknown number and size. Hypermutation, in certain bacterial systems at least, has thus a controlled temporal parameter based on the addition of genetic elements.

Mutator systems in various organisms other than bacteria, such as in a certain fungus and in the Ac–Ds system in maize, have transposition elements controlling the timing and degree of mutation [9,10]. This timing in the fungus enables an early hypermutagenic responsiveness to the environmental stress of high temperature. In the nuclei of this fungus, a genetic segment (or segments) become(s) deleted from a genetic complex and apparently becomes incorporated into a second complex. At a specific time, a contingent hypermutation then results. If the first deletion and subsequent incorporation do not occur, mutation within the second complex is far less frequent and considerably delayed [10]. This would greatly curtail a potential, adaptively responsive mutation to a temperature stress. This fungal mutator system is strikingly similar in behavior to the Ac–Ds controlling element system in maize. This may point to a common underlying process, if not origin. These two-part, mutator systems may have ultimately evolved from the type of bacterial, dual mutator system in which P1-type transduction played a critical, temporal role [11].

The adaptively responsive mutator processes in other bacterial systems might have transposons in a temporal role. In this regard, various adaptively responsive mutation systems were constructed to contain Tn and P1 virion elements in the genetic make-up of the bacteria. However, these elements or their effects were not the subject of investigation [5–7]. For example, in various cases, P1 virions were used to introduce, through transduction, particular genes into the bacterial genome. It is quite possible that the incorporated Tn and P1 genetic elements played an undetected, yet significant background role in the temporal promotion of adaptive hypermutation in these systems. In some situations, they, undetected, may have been responsible for a significant delay in or even a repression of a hypermutation, thus affecting adaptiveness.

To underscore this point, the presence of the Mu transposition element in a bacterial genome enabled an adaptively responsive mutagenesis in a particular bacterial system over an extended period [1,8]. However, when a second Mu element was incorporated into the genome, adaptive mutation did not occur. It was repressed [1]. This Mu system might point to type of aberrant, temporal control. Namely, an extra transposon delays, for an excessively long period, the occurrence of an adaptive mutation rather than hastens it. Interestingly, the Mu element was introduced through the integration of a lambda prophage into the chromosome of this bacterial system, that is, by a type of transduction. Also, the gene that was to be controlled by adaptively responsive mutation, involving two operons, had originally been introduced via P1 transduction [12]. One wonders what background role such transduction could have eventually played in this mutator situation. What would happen if such adaptively responsive mutator systems in bacteria were subsequently transduced with P1 virions? Would adaptively responsive hypermutation occur in particular, temporal patterns. If so, would it globally involve many or few cryptic genes? Experiments could answer these questions.

Do the temporal features of hypermutation in themselves enable us to better understand hypermutation or its basis? Its temporality strongly suggests that

hypermutation is a reflection of a developmental system. In such a system, the genome would fold into or upon itself at particular periods, due to incomplete force configurations being generated at those times through transposon incorporation and stress. Further conformational changes, involving different levels of organization, would necessarily ensue. Within such, otherwise distant genetic regions would have become variously connected with the aid of recombinational repair enzymes and the addition of more transposons. This would have created new, completed, functional adaptive genetic organizations at particular periods. Adaptive, dynamic completions would be thus enabled and created throughout the genome at critical times. Programmed hypermutation would be the manifestation.

This development would predictably give rise to a global rearrangement of genetic linkage relationships and changes in gene boundaries at certain periods. A global, temporal program according to which certain recombinational repair enzymes operate should also occur and be detected. Global changes in DNA sequences should also be a consequence. Investigations to test these experimental predictions could be undertaken. Computerized tomography could also provide visual evidence of a morphologically changing genome in which distant genomic regions are necessarily brought together at particular periods. These periods could be correlated with the onset of hypermutation and particular, adaptively functional, global genetic changes. Through which, a non-linear integration of various, distant cryptic regions would have occurred, and be detected. The ultra-high occurrence of concurrent mutation suggests the necessity of this integration and breakdown of genetic boundaries [11]. In fact, earlier research points to the fusion of different operons as a basis for adaptive mutation to a lactose medium [8].

If the results of such proposed investigations enable us to better understand hypermutation, especially its temporal features, this could lead to a more complete knowledge of the basis of gene function within mammalian development. From such, would come new, effective approaches in the treatment of genetically based diseases.

References

[1] J. Cairns, et al., The origin of mutants, Nature 335 (1988) 142–145.
[2] M.M. Lieber, T. Persidok, Mutability in *Escherichia coli* K12 enhanced by a P1 plasmid and by generalized transduction, Riv. di Biol. — Biol. Forum 76 (1983) 493–499.
[3] M.M. Lieber, New developments on the generation of mutations in *Escherichia coli* lysogens, Acta Microbiol. Hung. 36 (1989) 377–413.
[4] M.M. Lieber, Mutagenesis as viewed from another perspective, Riv. di Biol. — Biol. Forum 83 (1990) 513–522.
[5] B. Hall, Adaptive mutation that requires multiple spontaneous mutations. I. Mutations involving an insertion sequence, Genetics 120 (1988) 887–897.
[6] B. Hall, Spontaneous point mutations that occur more often when advantageous than neutral, Genetics 126 (1990) 5–16.
[7] B. Hall, Genetics of selection-indiced mutations. I. uvr A, uvr B, uvr C, and uvr D are selection-induced specific mutator loci, J. Mol. E 40 (1995) 86–93.
[8] J. Shapiro, Observations on the formations of clones containing araB-lacZ cistron fusions, Mol. Gen. Genet. 194 (1984) 79–90.
[9] B. McClintock, Chromosome expression and genic expression, Cold Spring Harbor Symp. Quant. Biol. 16 (1951) 13–47.
[10] M.M. Lieber, The genetic instability and mutagenic interaction of chromosomal duplications present together in haploid strains of *Aspergillus nidulans*, Mutat. Res. 37 (1976) 33–66.
[11] M.M. Lieber, Environmentally responsive mutator systems: toward a unifying perspective, Riv. di Biol. — Biol. Forum 91 (1998) 425–458.
[12] M. Casadaban, Transposition and fusion of the lac genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu, J. Mol. Biol. 104 (1976) 541–555.