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Adaptively responsive hypermutation and its configurational-based regulation due to global position effect

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Various related mechanisms have been proposed to explain the different types of adaptively responsive hypermutation [1,2]. More recently, it was argued [3] that a global regulatory gene in *Escherichia coli*, implicating a transposition element, controls the adaptively responsive mutability of the *ebg* operon. This operon is responsible for the utilization of a particular carbohydrate.

Relevantly, closely linked to this operon are two genetic regions, themselves operons, that determine or control maltose and xylose utilization. Mutant genes of these, *mal* and *xyl*, result in an inability of the bacteria to utilize maltose and xylose. Several years ago, the author found that ultra-high mutability to Mal^+ and Xyl^+, that is, to maltose and xylose utilization capabilities, occurred in bacteria on respective media separately containing these sugars as a sole carbon source [4]. The degrees of these mutabilities were generally uniform with respect to one another, suggesting a coordination of mutation related to the nutritional environment. Evidence indicated that transposons from a mutant P1 plasmid, contained in the bacteria, had integrated into the bacterial chromosome, and that such transposons were responsible for these and other coordinated, extremely high mutabilities involving many other genetic regions. Interestingly, *strR*, determining streptomycin resistance, located near *mal* and *xyl*, underwent a high frequency of associated mutations to a mutant gene determining streptomycin sensitivity. Bacteria which carried the *mal, xyl,* and *strR* genes and which lacked such transposons did not undergo mutation to Mal^+ and Xyl^+ while on maltose and xylose media, nor did they become streptomycin-sensitive.

Recently, I compared these extremely high mutabilities of *mal* and *xyl* that had occurred under stressful, selective conditions with the known spontaneous mutation rates, under non-selective conditions, of genes in *E. coli* responsible for an inability to utilize various carbohydrates. One found that collectively the mutations to Mal^+ and Xyl^+ under selective conditions had to have occurred to a far higher degree (at least 5×10^7 fold) than would have been expected had such mutations occurred collectively in non-selective, non-stressful conditions. An evoked mutator process, implicating transposons, had to have been most probably adaptively responsive or linked to the stress of these particular nutritional environments. Did this involve some unknown regulation of mutability, involving some type of global regulatory gene?

Such high mutability of *mal* and *xyl* was just one feature of a global hypermutation system in which many mutations, not adaptive in themselves, occurred throughout the *E. coli* genome in close association or coordination with those mutations adap-

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tively responsive to particular nutritional stresses [4–6]. This global coordination suggested that mutations not apparently adaptive in themselves were nevertheless necessary for the occurrence of mutations that were advantageous [4–6]. Subsequent research by Torkelson et al. [7] demonstrated a genome-wide hypermutation, involving three replicons in *E. coli*, in which many non-adaptive mutations occurred in coordination with those adaptively responsive mutations to a nutritional stress. A pattern of genome-wide hypermutation, very similar to the one described by the author, was clearly demonstrated as underlying adaptive mutation in this system. One cannot really explain such patterns of global hypermutation merely in terms of the effects of a global regulatory gene or genes.

The problem still remains of how to comprehensively explain such phenomena, which are clearly subject to some type of inner-directed, global regulation or control. In an attempt to do so, I proposed that such hypermutability could be explained by a type of non-local, global position–effect variegation (P–EV) inducing chromosomal instability by integrating genetic elements [4]. Classical P–EV does operate through non-local effects [8]. The integration of such elements or transposons into critical chromosomal positions, it was proposed, resulted in non-local, global, configurational changes in the architecture or topology of the chromosome. The global mutation that ensued was seen as a way of generating a stable, hence, completed, chromosome architecture or topology. In this connection, regulation of adaptively responsive mutability must, thus, extend to involve global changes in chromosomal configuration or conformation, and does not extend to some global regulator gene; unless, such a gene is a marker for a vulnerable genetic region, wherein insertion of a transposon always leads to a non-local, configurational change and a consequently engendered instability.

How, specifically, does an external environmental stress, in the form of nutritional stress, influence such configuration-based regulation of mutability? In bacteria, the particular nutritional stress itself would be seen as evoking particular, chromosomal configurational changes, non-uniformities or incompletions, through a type of dynamical imprinting; this would evoke the repositioning of genetic elements as a means to reestablish global stability within the genome. These conformational changes along with the configurational changes due to transposon integration and reintegration, involving aberrant recombination, itself configurational change, would result, however, in a further global instability or configurational incompletion within the genome. This would generate a global mutability defined by further configurational change, possibly involving intrachromosomal recombination. This would lead to a new, stable, possibly more completed, global chromosomal configuration. Such a new configuration, or particular part thereof, would enable a specific, stable accommodation to a particular stressor, perhaps marked by the global activation of a set of operons. Hence, one could attribute to such configurational changes, having involved repositioning, seemingly regulatory features of a gene with global effects. The above also shows how a mutator-dynamic, based on global configurational change involving rearrangements, is a necessary complement in adaptively responsive mutagenesis.

To better grasp this picture, one must realize that a genome is not just a collection of molecules, but is a complex architecture or configuration held together or stabilized by electromagnetic and mechanical forces. Genome configuration embodies force configuration. Furthermore, one must appreciate that a genome is connected to various scales of its environment by forces. Stressful or destabilizing changes in the form of non-uniform or incomplete force configurations within this genomic–environmental, dynamical system must lead to a restabilization, or greater stabilization, by means of a global, regenerative completion of force configurations within the system [6,9,10]. The addition, through recombination, of a transposon to a chromosomal region may be one manifestation of this completion process, and may very well be represented by operon activation. This is what would underlie the global regulation of adaptive mutation that occurs on various scales, but would be perceived by us as the apparent effect of a global regulatory gene or genes.

The replication of the genome itself is a regenerative, completion process, a dynamic completion of incomplete complements, one that preserves the genome through space–time. Thus, might hypermutation in response to internal and external stress be

an extension of this completion process that, within various scales of genomic architecture, preserves the integrity of the genome while in stress, and through space–time. An underlying, completive process is suggested. It is one requiring an inclusive interpretation of diverse phenomena.

The interface between a molecular–regulational interpretation of hypermutation and one based on the behavior of forces, within a genome, is configurational change of the genome and the patterns of such change. Regulation of adaptively responsive hypermutation, based on the consequences of particular configurational changes, unites the molecular–genetical and dynamical models of hypermutation. Research should focus on this interface.

In fact, through earlier research, there is evidence in lower organisms of transposon-based mutator processes implicating changes in chromosomal configuration with non-local effect (references in Ref. [4]). Relevantly, in higher organisms, gene regulation, responsive to environmental factors and stresses, such as temperature changes, is controlled non-locally by chromosomal configurational changes manifested by intense coiling and uncoiling of chromosomal regions, and detected cytologically as heterochromatin and euchromatin, respectively [11,12]. The non-local, genetic consequence of P–EV, itself, involves transposition near or within the particular spreading configuration that is heterochromatin, resulting in clonal variegation on the phenotypic level. Heterochromatin is known to be genetically unstable in a regulatory manner in certain developmental situations, with permanent clonal consequences, especially when nuclei are in certain cytoplasmic environments [13,14], possibly stressful.

Various types of mutator processes in different organisms could be explained by intrachromosomal crossing-over, within heterochromatin, triggered by the conformational changes of heterochromatization, upon and due to repositioning within the genome [15,16]. This view was used to explain a programmed, developmental genetic-instability system in a fungus very similar to the Ac-Ds, dual element-controlled mutability system in maize [16]. Later reinterpretation of data pertaining to this fungal system, which produced clonal variegation on the phenotypic level, suggested a programmed, developmental hypermutation on the chromosomal level, adaptively responsive to the stress of higher temperature [6].

Years ago, Barbara McClintock explained controlling-element based mutability in maize, which produced clonal variegation, in terms of a P–EV model implicating heterochromatin [17], and, later, she pointed out that the genome in higher plants rearranges itself, through programmed transposition, in response to stress [18]. The model I present to explain adaptively responsive, global hypermutation could be seen as a generalization of the P–EV regulation model presented by McClintock, and one that has developmental features. This model would predict clonal subpopulations of cells containing clusters of "non-adaptive mutations" linked with an adaptive mutation(s), and this is what Torkelson et al. in fact found [7]. Moreover, they provide evidence suggesting that this type of hypermutation involves recombination. Recombination, deriving from a higher-ordered configurational change, could very well be a means for completing the non-uniform, unstable force configurations within the genome.

If forces through their stressful, non-uniform configurations within the genome play a significantly regulatory role in promoting an adaptively responsive, completing-hypermutation, as manifested by a global P–EV, what situations could arise from this? For example, what would be the particular regulation of mutabilities if such a genome containing hypermutators in bacteria or yeast is exposed to low levels of electromagnetic forces or changes in inertial forces, or exist in minimal gravitational fields, while such organisms are exposed to nutritional stress? Would an adaptively responsive mutation occur, and if so, would it be enhanced, and, in what way? Would a particular chromosomal or genomic conformation be connected to this adaptation in particular subpopulations of cells? Experiments designed to answer these questions may lead to new perspectives or insights, and I welcome others to use the hypermutator system that I discovered [4] in order to investigate this and related matters.

Global hypermutation appears to be a fundamental process of regeneration in nature, hence, completion [6,10], and, thus it may be involved in the generation of cancer. Others have argued that hypermutation plays a role in carcinogenesis, e.g., Ref.

[19]. Several years ago, this author speculated that carcinogenesis is an imperfect attempt by an organism, using mutator-genetic elements, to evolve within its lifetime [20], the implication being, attempting to evolve regeneratively, adaptively in a healing manner, in response to internal stress. Can this be a deeper meaning of carcinogenesis?

Being a fundamental process, adaptively responsive global hypermutation may provide us with insights into an underlying unity or principle within the universe, and thus provide us with a deeper understanding of biological and physical phenomena, perhaps pointing to the possible drive of all phenomena to complete themselves through regeneration [6,21]. For us, the challenge is to strive for and use ever more completed paradigms in science, and this will require courage. We can do no less if science itself is to adaptively evolve.

References

[1] S. Rosenburg et al., Adaptive mutation by deletions in small mononucleotide repeats, Science 265 (1994) 405–407.
[2] S. Rosenburg et al., Adaptive mutation sequences reproduced by mismatch repair deficiency, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 12017–12020.
[3] B. Hall, Adaptive mutagenesis at ebgR is regulated by phoPQ, J. Bacteriol. 180 (1998) 2862–2865.
[4] M.M. Lieber, New developments on the generation of mutations in *Escherichia coli* lysogens, Acta Microbiol. Hung. 36 (1989) 377–413.
[5] M.M. Lieber, Mutagenesis as viewed from another perspective, Riv. Biol. — Biol. Forum 83 (1990) 513–522.
[6] M.M. Lieber, Environmentally responsive mutator systems: toward a unifying perspective, Riv. Biol. — Biol. Forum 91 (3) (1998) 425–458.
[7] J. Torkelson et al., Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation, EMBO J. 16 (1997) 3303–3311.
[8] E.B. Lewis, The phenomenon of position effect, Adv. Genet. 3 (1950) 73.
[9] P. Lieber, Aspects of evolution and a principle of maximum uniformity, in: C.H. Waddington (Ed.), Towards a Theoretical Biology II, University of Edinburgh Press, Edinburgh, UK, 1969, pp. 293–316.
[10] M.M. Lieber, Force, development, and neoplasia: development from another perspective as illustrated through a study of in vitro plant development from neoplasm, Riv. Biol. — Biol. Forum 89 (1996) 245–274.
[11] I.J. Hartmann-Goldstein, On the relationship between heterochromatization and variegation in *Drosophila*, with special reference to temperature-sensitive periods, Genet. Res. 10 (1967) 143.
[12] U. Goodeneough, R.P. Levine, Genetics, Holt, Rinehart, and Winston, New York, 1973.
[13] S. Beermann, A quantitative study of chromatin diminution in embryonic mitoses of *Cyclops furcifer*, Genetics 54 (1966) 567.
[14] J.A. Burns, D.U. Gerstel, Flower colour variegation and instability of a block of heterochromatin in *Nicotiana*, Genetics 57 (1967) 155.
[15] M.M. Lieber, The effects of temperature on genetic instability in *Aspergillus nidulans*, Mutat. Res. 34 (1976) 93–122.
[16] 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.
[17] B. McClintock, Chromosome expression and genic expression, Cold Spring Harbor Symp. Quant. Biol. 16 (1951) 13–47.
[18] B. McClintock, The significance of responses of the genome to challenge, Science 226 (1984) 792–801.
[19] A.A. Stark, Transient appearance of the mutator phenotype during carcinogenesis as a possible explanation for the lack of mini/micro-satellite instability in many late stage tumors, Mutat. Res.: Fundam. Mol. Mech. Mutagen. 421 (1998) 221–225.
[20] M.M. Lieber, Mutation, development, and evolution, Thesis, Institute of Animal Genetics, University of Edinburgh, 1967.
[21] M.M. Lieber, The living spiral. A dimensionless biological constant gives a new perspective to physics, Riv. Biol. — Biol. Forum 91 (1) (1998) 91–118.