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Environmentally Responsive Mutator Systems: Toward a Unifying Perspective

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Abstract. *Biology has long sought a unifying principle. The behaviour of genetically controlled mutator processes adaptively responsive to stress may reflect such a principle. Related mutators exist in diverse organisms from bacteria to mammals. Such systems have evolved from one another and have defined the very evolution of organisms. Many of these mutator systems can determine in a developmental manner a ultramutability or hypermutability throughout the genome. Though the genetic control of high levels of mutability can reflect molecular features, such mutagenic processes reflect a deeper parameter involving forces and their configurations. These configurations*

must generate stable or uniform configurations from unstable ones throughout the genome and organism. Directed mutation becomes a generative process attuned to non-uniform forces of local niches and to the more uniform forces of a universal niche. The manner of mutagenic, attuned response depends on the level of genomic and transgenomic organization. This is reflected in hierarchies of evolution. Directed mutation is a feature of a universal, generative ordering process, and this feature is marked by a universal dimensionless constant. It is the dynamic consequence of such directed generation which ultimately confers adaptation through dynamic completion. This suggests an underlying, unitary, and necessary dynamics connecting ultramutability systems in all organisms and would serve, in complementation with a molecular approach, to elicit new and productive research avenues. One outcome would be the illustration of a unifying principle governing biological and physical phenomena.

1. INTRODUCTION: MUTATOR PROCESSES AND A UNIFYING PRINCIPLE

Is biology able to find a unifying principle for itself? From the standpoint of genetics, such a principle may be manifested through the behaviour of mutator systems apparently responding adaptively to stresses ultimately in the form of non-uniform forces. Through the years, mutator systems have generally been dismissed by genetics textbooks as being aberrant, exceptional cases to the standard, basic behaviour of mutation processes. However, more and more evidence has been accumulating to show that mutator systems, and their significance for development and evolution, may not be exceptional situations after all. Such adaptively responsive mutator processes may throw light on what mutation and evolution really mean or reflect.

Mutator processes, via the genetic determination or control of mutagenesis, have been very significant parameters in biological development and evolution. In the very evolution of developmental systems, the mutator processes themselves could have become features of development (Lieber [1967], [1972], [1975], and [1976b]). This would of course also apply to the evolution of the immunological system. In the very functioning of immunological systems, a mutator process, through a genetically regulated hyper-

mutability, has proven to be a significant component in the generation of immunological diversity in response to an infective antigenic stress (see Steele & Pollard [1987]; Steele *et al.* [1997]; and Borst & Greaves [1987]).

As mutator processes have been significant components in the evolution of developmental systems in all biological organisms, one would expect variations of the type of genetically controlled and environmentally sensitive hypermutability manifested in mammalian immunity to be exhibited in the genome of lower organisms such as bacteria and fungi, and in other organisms such as higher plants. Being less complex organisms, a more general, less differentiated hypermutability would be predicted as involving the genome of those less evolved organisms. Moreover, the level of organization of the genome involved would help to define the degree and pattern of mutation generated. While describing and linking pertinent research on hypermutability, this article will illustrate this and its significant ramifications, especially its relevance to higher organisms. In so doing, it will approach a unifying view on mutability and development that provides a deeper insight into evolution itself, and one that points to a unifying principle in biology.

2. GENETICALLY CONTROLLED GLOBAL MUTAGENESIS IN LOWER ORGANISMS: BACTERIA

A global, genetically controlled and environmentally responsive mutagenesis involving the entire genome was discovered in particular *Escherichia coli* strains (Lieber and Persidok [1983]; Lieber [1989], [1990]). In such *E. coli* strains, transposition elements from a mutant plasmid, referred to as P1CMrec, were incorporated into the bacterial chromosome generating new mutant genes. Some of these genes conferred additional and particular auxotrophic requirements. Such incorporation also generated a high mutability of widespread genetic regions consequently leading to the production of prototrophic mutants. In addition to those existent transposition elements, such strains subsequently incorporated segments of bacterial DNA into their chromosomes via a

generalized transduction mediated by P1 virions. As a consequence of this, the degree of mutation, compared to non-transduced mutator strains, was greatly, uniformly, and globally enhanced throughout the genome in those transduced mutator strains on selective media, making this a two-part or two-stage mutator system with global, ultramutagenic effects.

Though involving widely separate regions in the genome, such mutations occurred simultaneously, as though linked, and to the same uniform degree. Evidence suggested that their frequency of occurrence was also tied into the selective growth media, though in an indirect manner. In accord with the data (see Lieber [1989], [1990]), the frequency of occurrence of any unselected mutation to a given prototrophy (or prototrophies), in this two-part mutator system, is tied to the occurrence of those mutations to various prototrophies enabling adaption to growth medium lacking certain nutrients. The generation of mutation to unselected prototrophy was necessary for, or a necessary precursor to, a near-subsequent or concurrent mutationally adaptive response on the part of various genes to other selective, nutritional, hence starvational, stresses. In various cases, from three to six different prototrophic mutations were concurrently generated in a unified, adaptive response to the lack of given nutrients in the medium, that is, to the joint stresses of starvation. These phenomena were repeatable many times, and it would be predicted that related phenomena would be found in other, related bacterial systems with mutator properties highly sensitive to environmental stresses.

Hall [1995] shows that in *E. coli* starved for proline there is a burst of mutations from tryptophan auxotrophy to tryptophan prototrophy when proline starved cells are plated onto a medium lacking tryptophan but containing proline, hence non-selective for proline prototrophs. However, he did not test to see whether such *try* prototrophs became originally also *pro* prototrophs. He concludes that such proline starvation produces a state of cellular stress giving rise to a hypermutation leading to the adaptively responsive *trp* mutability. He also points out that the degree of such is enhanced in the presence of repair-deficient markers.

In the P1CMrec system, *rec* is a genetic marker for a deficiency in recombination/repair. Its likely transposition from the P1 plas-

mid into the bacterial chromosome may have also been involved in the global enhancement of genetic mutability throughout the chromosome; or, from another perspective, it may have been involved in a controlled generation of an adaptively responsive ultramutability to environmental stress subsequent to, but dependent on, generalized transduction.

Recently, this author re-interpretated his original data (Lieber [1989]) bearing on the *E. coli* dual P1CMrec-mutator system while considering the fact that the general spontaneous mutation rates in *E. coli* are 10^{-8} to 10^{-10} when such bacteria are under non-selective, non-stressful conditions (Goodenough & Levine [1974]; Bates *et al.* [1989]; Hall [1988], [1995]). Such inclusive re-interpretation has provided further and broad support for the presence of an adaptively responsive, though internally regulated, ultra-mutability-system involving transduction. In some cases, when compared to the spontaneous mutation rates under non-selective conditions, the concurrent generation of mutation in the *E. coli*-P1CMrec-mutator system to various prototrophies under selective conditions was of an order of 10^{27} over what would have been predicted if such mutations to various prototrophies were to have occurred at random, that is, independently from one another and from any environmental influence. Such an ultra-high order of magnitude dramatically indicates that a transduction-mediated mutation process, implicating controlling elements and possible repair-deficient markers, was adaptively and globally responsive to a selective, nutritional stress. Such mutation would thus appear to have regulatory, developmental features sensitive to changes in local environmental situations.

This mutation-mediated, generative process is far higher and far more encompassing than those apparently adaptively responsive mutation processes that have been described by Cairns [1988], Hall [1988], [1990], [1991], [1995]) and Shapiro [1984]; though recently, Torkelson *et al.* [1997] point to a genome-wide hypermutation, involving many loci, in a sub-population of bacterial cells that *underlies* recombination-dependent adaptive mutation. Yet, significantly, this underlying hypermutation or its underlying mutator process is not completely elucidated. Polymerase-mediated misrepair of stress-induced DNA breaks is proposed by this

group as contributing to global hypermutation. But, why would stress-induced DNA breaks occur in the first place? This explanation requires implicitly that forces via the environment are changing DNA structure. Furthermore, the polymerase misrepair activity is in effect a dynamic re-stabilizing of the structure or configuration of DNA, i.e., generating genomic DNA of stable or more stable dynamical configuration that is molecularly marked by changes in nucleotide sequences, changes that are necessary for such global dynamical stabilization. Clearly, a molecular explanation for hypermutation requires implicitly the global operation of forces in various stages and in various dynamical patterns. By not acknowledging and elucidating such underlying and subsuming dynamics, molecular explanations would have to be incomplete. Might this be due in general to the limitation of current molecular models in genetics?

How could such an encompassing mutator process have been manifested in the P1CMrec-mutator system, even in light of a limited molecular explanation? Such a process would have had to occur in every bacterial cell and involve most, if not all, of the genetic regions of every bacterial genome. Those functional, regional boundaries within respective genomes would have had to be changing, as well, so as to have generated new functional, regulatory genetic regions. The fusion of different cistrons leading to adaptive mutation in *E. coli* through transposon involvement (Shapiro [1984]) would be evidence of or a marker of an aspect of such. This widespread or global phenomenon would suggest a universal, directed mutagenic process, regulatorily attuned to environmental stresses, that must occur in all organisms in various ways and on various levels of genomic organization. It is a process whose elucidation must require new, more inclusive perspectives, and hence new predictions and experimental approaches.

3. GENETICALLY CONTROLLED, ENVIRONMENTALLY RESPONSIVE MUTAGENESIS IN PLANTS IS RELATED TO THAT OF BACTERIA

Such a directed, mutagenic process would, predictably, occur

on the chromosomal or karyotypic level of genomic organization in various species. In the fungus *Aspergillus nidulans,* a lower plant, temporally programmed or ordered deletions occur from a chromosomal complex (Lieber [1976b]). This programmed, genetic control of mutation at the chromosomal level in a specific chromosomal region is under the direction of another complex mutator element/structure located at another chromosome. This two part, regulatory mutator system operating at the chromosomal level in *Aspergillus* is very similar to the two part, Ac-Ds mutator, controlling element system which generates deletions from chromosomes in an orderly manner in maize, a higher plant (McClintock [1951]). The two part, programming mutator-controlling element system in *Aspergillus* represents the first of its kind discovered outside of a higher plant, and this suggests that the type of mutator system in maize may have evolved from the type exhibited by a lower plant such as *Aspergillus.* (The importance of the *Aspergillus*-mutator system was acknowledged by B. McClintock in a personal, written communication in 1976.)

This Ac-Ds type mutator system in *Aspergillus* represents a type of localized, yet transposable, controlled hypermutability at the chromosomal level of organization. More important, it may represent a chromosomal hypermutability, or deletional instability, adaptively responsive to local environmental stress through a genetic regulation connected to such stress. This hypermutability in *Aspergillus* is manifested phenotypically in the concurrent production of many yellow sectors of improved growth rate from green, haploid colonies of restricted growth rate. This, in itself, represents a mutator controlled and based differentiation, and one analogous to the mutator generation of diverse, mutant clones underlying immunological diversity.

A local temperature stress can significantly and greatly increase the frequency of such yellow improved sectors (Lieber [1972], [1976a], [1976b]) (Fig. 1). Under the given temperature stress, the generation of yellow sectors of improved growth rate and spore production, through programmed deletion, might very well be an adaptive response, on the part of *Aspergillus* colonies, to a temperature stress, i.e., to a radiant-heat stress. Certainly, improved growth rate and spore production would have beneficial, adaptive

features or implications, and these features would be transmitted to subsequent generations by means of the pigmented, a-sexual spores (conidia) produced by the sectors. Hence, it could be concluded that a genetically controlled or programmed hypermutability in *Aspergillus* on the chromosomal level is a controlled, yet connected, adaptively mutagenic, and inheritable, response to a local stressful environment, namely a stressful, radiant-heat condition based upon infrared, electromagnetic radiation.

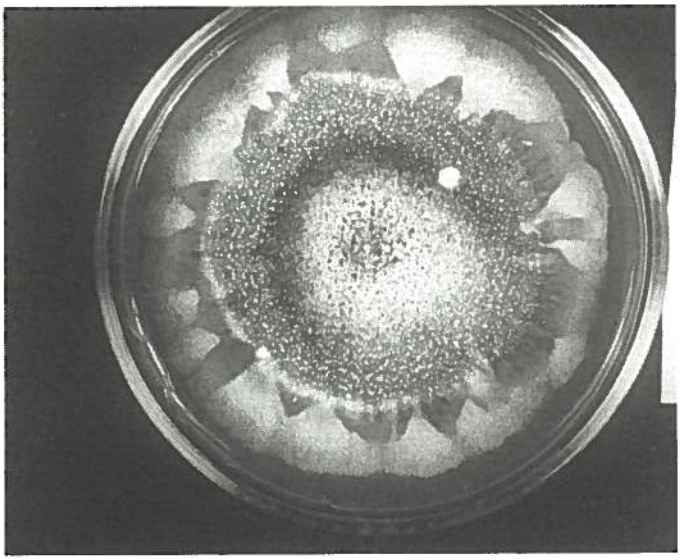

Fig. 1 – A colony of *Aspergillus nidulans* from a group having produced many mutant yellow sectors in response to a temperature stress. The improved morphology, growth-rate, and conidial production of such sectors would now suggest an adaptively responsive, inner-directed mutagenesis to temperature stress (from Lieber [1972]).

In *Aspergillus,* a more inclusive adaptive response can take place that mutagenically involves the karyotypic level of organization of the entire genome. Normally, *Aspergillus nidulans* is a haploid organism. However, green diploid strains of such can be isolated and generally remain stable as such. Occasionally, they undergo haploidization in which chromosomes are lost successively until a

stable haploid state is reached and is manifested as a haploid sector produced by a diploid colony (Pontecorvo *et al.* [1953]). Under non-selective conditions, the production of such haploid sectors is infrequent. When diploid colonies are cultured on media containing the growth inhibitor DLp-fluoro-phenylalanine (pFA), the growth or growth rates of such diploid colonies is greatly reduced or stunted; on such media, these colonies are light brown with a grooved morphology, producing no green conidiophores which are the normal, a-sexual vegetative reproductive structures. However, the production of haploid sectors from diploid tissue is greatly increased on such media (Morpurgo [1961]). Most important, these sectors are green and smooth, producing abundant conidiophores and are of greatly improved or normal growth rates.

By these criteria, such haploid sectors are clearly adaptive to the growth inhibitor. Though this method of inducing haploidization using a growth inhibitor was used as a routine device by *Aspergillus* workers to ease the isolation of haploids from diploids for subsequent genetic tests, the deeper implication of induced haploidization was not seen. Now it can be readily seen: Such induced haploidization represents an adaptively responsive genomic or karyotypic instability, involving the entire diploid karyotype, to a local, environmental stress, namely a growth inhibitor. The genomic consequence of this haploidization is readily and quickly transmissible to subsequent generations in similar conditions by means of abundant, a-sexual spores produced by the green pigmented conidiophores on the sectors.

Relevantly, the colonial green algae, *C.eugametos,* exhibits under stress a phenomenon, one can interpret as an adaptively responsive mutagenesis, that enables growth in the presence of a growth inhibitor similar to pFA. The discoverers of this phenomenon (McBride and Gowans [1969]), however, do not interpret this phenomenon as being an adaptively responsive mutagenesis to a selective agent but due to their growth inhibitor exhibiting mutagenic properties in their experiments. However, this interpretation strangely misses the implied and significant issue in this type of situation; namely, a local, environmental stress, whatever its form, leading to an adaptively responsive mutagenesis would itself, in such a context, have to be a mutagen or take part in a mutagenic

manner. Thus, our concept of what is a mutagen would have to be broadened. Significantly, McBride and Gowans point out that the high frequency of beneficial mutation they noted in relation to the presence of the growth inhibitor, as opposed to its non-presence, may have been to due the growth inhibitor inducing controlling elements similar to those responsible for the controlled genetic instability in maize studied by B. McClintock ([1951], [1965]).

McClintock [1984] pointed out that frequent genomic reorganization, in maize and in other organisms, can occur under conditions of environmental stress and indicated that the dogma of a constant genome must be reconsidered (McClintock [1980]). In the P1CMrec-*E. coli* system, which could be a more primitive, two-part mutator version of the dual mutator system in *Aspergillus*, in the *Aspergillus*-system itself, and in maize, the high degree of genetic change, or hypermutability, involves the incorporation and release of genetic elements in a stressed genome. Such release and incorporation would involve recombination-type events sensitive to stress.

Because in higher and lower plants there is no real difference or barrier between somatic and germ tissue, the adaptive genomic consequences of hypermutability could readily have been transmitted to progeny within necessarily short periods. The results would have been rapid, major adaptive evolutionary change. These inner-directed, though environmentally responsive, mutator-systems would have provided the inner direction to evolution so as to have made it more responsive, and hence flexible.

4. ADAPTIVELY RESPONSIVE GENETIC INSTABILITIES REFLECT A MEANS TO RESOLVE THE NON-UNIFORMITY OF FORCE IN STRESS

As argued elsewhere (Lieber [1989]), the global genomic changes mediated through the P1CMrec-bacterial system involved creation of global changes in the *E. coli* chromosome brought about by the incorporation of genetic elements in two stages, through recombination. Such changes were seen as being non-uniform force configurations generated throughout the chromosomal

genome. Such non-uniform force configurations made the genome dynamically unstable, posing an internal genomic stress. This internal stress would enable the genome, by virtual of its dynamical instability, to globally respond adaptively to a concurrent stress from the local environment, as well. Genetically controlled, global mutagenicity was seen as an expression of the dynamic process restoring the original chromosomal stability or promoting an even greater stability (Lieber [1990]).

Intrachromosomal recombination events, the release of transposition elements, and directed misrepair, are all connected processes used to account, directly or indirectly, for high mutability (e.g. Rosenberg *et al.* [1995], [1997]; Steele *et al.* [1997]; Hall [1991], [1995]; Borst & Greaves [1987]; Federoff [1984]; Walker [1977]). They would be the molecular and genetic markers of such an underlying generation to greater genomic stability. The new, interrelated mutations would be the markers of this greater dynamic stability established within the entire genome. Aberrant intrachromosomal recombination, the release and incorporation of various types of transposition elements, and the related induction of the various repair mechanisms would be seen as the molecular means (or manifestation) for establishing a highly stable, and hence uniform, force configuration throughout a genome and between the genome and its cellular and external environment, leading thereby to the phenotypic adaptability of the organism.

This means that the transduced genome of the P1CMrec-bacterial system must respond mutationally as a co-ordinate unit or integrity to stress in order to have the bacteria accommodate to a specific stress or stresses, whether due to external or internal factors, or both (Lieber [1990]). Such specific stress would be related to genetic element incorporation and also involve, in a synergistic manner, a dynamic imprinting from an environmental stress; this would be a non-uniformity of force intersecting with and imprinting on the genome. Responsive global mutations, being a unitary, co-ordinate response (or generative resultant) to such stress, which, in themselves, may not appear to be related to the specific stress in question, would nevertheless and necessarily lead to, or be needed for, those co-ordinate genomic changes that are adaptively responsive.

Particular globally configurational changes in the genome, due to dynamic stress from the environment and from initial genetic restructuring, would generate global ultramutability leading to other, specific chromosomal configurational changes that globally allow the generation of adaptively responsive, specific mutability via mutator processes. Global ultramutability, based on global configurational changes, would set the genome to configurationally differentiate into specific unstable states or mutant stages. Though not in themselves directly adaptive, these stages would yet provide the necessary dynamic channels within the genome leading to specific, adaptively responsive mutability. Hence, the latter becomes a derivative, developmental extension of global mutability. In this context, mutagenicity becomes a generative, developmental feature of the genome. It reflects the genome as a responsively developing entity.

Those mutations not obviously adaptive in themselves could nevertheless lead to phenotypic developments not apparently linked to local demands. Such "mutations or hypermutations could also help the expression of [adaptively neutral] concealed traits of organisms, thus increasing variability and complexity" (Sermonti, personal communication). Hence, an ongoing increase in such variability and complexity in this situation would be evolutionary changes not in themselves necessarily adaptive. They would, however, be developmental, phenotypic by-products, adaptively neutral, leading indirectly and necessarily to those other developed features that give accommodation to a niche. This is an important possibility to consider when evaluating the deeper implications of hypermutation in various organisms, including bacteria, and one various investigators of hypermutation appear to ignore.

In this connection, Hall ([1990], [1991], [1995]) contends that high, environmentally induced mutability in his bacterial system leads to hyper-generation of different, environmentally non-specific, unrelated mutations, some of which are later selected in a sieve like manner on selective media. However, the global, concurrent and linked hyper-generation of many different prototrophy-conferring mutations in the two-part P1CMrec mutator system of *E. coli* is not in accord with that hypothesis. Moreover, other than

invoking the concept of induced cellular stress, Hall's account does not explain why nutritional stress should lead to widespread mutation in the genome in the first place. In an earlier article, Hall [1988] nevertheless, presents a different view, not invoking the concept of hypermutation, which does suggest the significance of concurrent mutations involved directly and much less obviously in an adaptive response.

5. ADAPTIVELY RESPONSIVE MUTABILITY IN HIGHER ORGANISMS ALSO POINTS TO A MEANS TO RESOLVE GENOMIC STRESS

An adaptively responsive mutagenicity to environmental and genomic stress would require precise dynamical connections from those forces holding the genome together to those forces composing the various environments of the genome. This would especially have to be the case in the generation of immunological diversity. Such generation is based upon a highly specific, regulated, and directed mutagenicity responding to antigen-induced stress, as described by Watson *et al.* [1983], and Borst & Greaves [1987].

Steele *et al.* [1997] propose a mechanism for an antigen-generated or driven hypermutability of immunoglobin genes in mice. This hypermutability, according to Steele *et al.*, would have its source in a reverse-transcriptase, RNA based mutator system involving a structure he refers to as a mutatorsome and one associated with cell division. This system, his model implies, would have to be highly responsive in a specific, mutagenically adaptive manner to antigenic stress. Yet, his model does not define how this would be so. How does such stress convey immunological information so as to have this mutator system respond in a specific, precise immunologically adaptive manner?

The testable explanation to this may ultimately point to environmentally induced changes of force configurations within complex molecules such as DNA, m-RNA, and repair and replication enzymes. The genome would be seen as being joined to or intersecting with the nuclear membrane for this to happen, and predictably, there are interactions, at attachment sites, between chro-

matin and the nuclear membrane in certain insects and amphibians (Marshall *et al.* [1996]; Murray and Davies [1979]). Such a membrane is continuous with other membranes of the cell. Thus, such induced changes, and their necessary precision, would have to be generated through a process of dynamical imprinting conveyed via membranes of the cells to the chromosomes after such membrane exposure to antigen-imprinting.

Imprinting would have to involve the non-linear, dynamic intersections of structures. From such imprinting, via topological changes in membranes and other organelles of the cytoplasm, would ensue specific though destabilizing changes in molecular and chromosomal configuration, thereby determining specific changes in genome stability, template recognition, genetic repair and genetic replication, leading to a specific accommodating hypermutability. Such hypermutability, having molecular features to be sure, would nevertheless result in a greater global stability of forces throughout the genome than had existed before the stress-induced destabilization.

In the adaptive evolution of organisms, mutator systems based on such dynamic imprinting, via membrane-systems connecting the internal environment with that of the external, may have played a significant role. They may have themselves evolved into complex structures from those mutator-ultramutability systems represented in *E. coli* and *Aspergillus*. During evolution, mutator systems may have been generated in somatic tissue through chromosomal reorganization (Lieber [1972], [1976b]). These mutators, and their induced genomic modifications, may have been transferred to the germline in animals, and hence to subsequent generations, by way of a reverse transcription process, and thereby enhanced the inner direction and rate of evolution (Lieber [1967]).

Significantly in this regard, Gorczynski and Steele ([1980], [1981]) have repeatedly shown that frequently induced beneficial mutations in antigen genes in the somatic tissue of mice can be transmitted to progeny and proposed this has occurred through a DNA-RNA-DNA reverse transcription process involving a retrovirus, in a symbiotic role, carrying genetic information from the soma into the germline. This very important phenomenon and its possible basis would be a significant example of an adaptively

responsive mutator system (involving a type of transposition element), extended in space-time, which in the very exercise of its adaptive function is transmitted to progeny.

These adaptively responsive hypermutability systems that Steele and his colleagues describe, the first to be noted in a mammal, are very relevant to those examples of globally adaptive mutagenesis, highly sensitive to local, environmental stresses, that have been found in lower organisms. Those systems in a mammal, probably more refined and differentiated, may have in fact evolved from those ultramutability systems in lower organisms in that they may represent an evolutionary denouement from earlier types of mutator systems exhibited by lower organisms. The adaptively responsive generator of diverse immunoglobin and antigen genes thus points again to the universality of a mutator-driven, adaptively responsive mutagenesis as an inner dynamics of evolution, and so having determined the very course and rate of that evolution. It may suggest that evolution in mammals, especially the evolution of immunological-related systems, is not ultimately based on random events.

6. DEVELOPMENTAL MUTAGENESIS REFLECTS AN INNER, UNDERLYING ADAPTIVELY UNIFYING DYNAMICS

Mutator driven processes, though marked on the molecular level as, for example, DNA instability, its controlled misrepair, and related aberrant recombination, must ultimately be based upon and expressed through forces, such as mechanical and electromagnetic (EM) forces. EM forces interacting or intersecting with DNA do possibly lead to changes in DNA configuration, a promotion of DNA destabilization, and a related induction of transcription (Blank and Goodman [1997]). Moreover, the EM and mechanical forces are the forces that link by way of various cellular organelles, such as internal and boundary membranes, the genome of cells to the "external environment". Other biomolecular processes/structures would be connected to these membrane complexes. As seen in the case of stress and hypermutability, the nature of this connection would have such processes and structures

dynamically imprint themselves on such complexes. The former in turn would be imprinted upon by the forces embodying the membranes.

The resultant biomolecular processes/structures, involved in such mutual dynamical imprinting through intersection, would be manifested by, for example, changes in DNA and RNA configuration/behaviour. They would also be manifested by changes in the conformation, and thus, behaviour of repair, replication and translation controlling enzymes. Yet, the significant role that forces play in changes in biomolecular behaviour, and thereby in the generation of hypermutation, has not been acknowledged.

Many molecular explanations of hypermutation generally involve misrepair of DNA, leading to very small deletions, within aberrant recombination (reorganization) triggered by nutritional stress (e.g. Rosenberg *et al.* [1994], [1995]). In the context of a model which emphasizes the primacy of force in mutation, nutritional stress as non-uniformity of force is seen as imprinting on DNA, creating non-uniformity in DNA configuration which, at times, could very well be reflected as DNA breaks. Aberrant recombination through misrepair enzymes (polymerases) would generate a greater uniformity of forces within such DNA, through a re-completion process (i.e., the adding of new nucleotide sequences), hence generating a greater DNA stability. Thus, molecular features of hypermutability are markers of a dynamics which necessarily generates increase of stability within the genome through global, dynamic complementation or completion. This complementation is seen as necessarily completing the non-unformities generated through imprinting. Because it would be completing specific non-uniformities, in a sense being guided by them, such dynamical completion would have to be precise or specific.

In this regard, DNA replication, m-RNA creation through transcription, and protein synthesis through translation of m-RNA become ongoing processes of complementation or completion. These are processes that would maintain biomolecular stability or cohesiveness throughout the cell before and after dynamical imprinting. Such dynamical completion, generating stability, would be the deeper purpose or function of such processes and the ex-

pression of a deeper informational code. Dynamic, specific completion would be essential for functional integrity itself, and, in this context, causation and dynamical completion would become one in the same. On a higher level, mitosis would reflect this ongoing dynamic completion. Hence, growth or regeneration becomes a complemention process that is an ongoing functional accommodation to stress, and thereby a type of mutation at a higher level through which integrity is maintained.

On the genomic level, genetically directed, global mutation responding to stress, and its important features in many cases, such as the insertion and removal of transposons from chromosomes, become various ways of re-structuring a genome, through a complementation process, toward a organization that has greater dynamical stability. This would be a stability marked by a greater uniformity of force within a diverse genome. This could be manifested as the necessary establishment of more coherent domains within the genome. Within modern day species, most, if not all, mutator processes, including the two-part mutator system in *Aspergillus,* have been induced by genomic reorganizations on various levels (Lieber [1976b]). These reorganizations would have created non-uniformities of force within the genome. Whether such reorganizations are due to external or internal factors, or most likely both, the directed mutation process (or mutator situation) is elicited, through the earlier reorganization, to globally establish a subsequent, genomic organization having the greatest uniformity of force within a diversity of force.

Such a dynamic accommodation within and to an inner environment would be expressed phenotypically as an intra-organismic adaptation, an increase in the integrity of the organism, with evolutionary implications. Some of the phenotypic traits attendant to such inner accommodation of the genome with itself would appear, however, to be adaptively neutral with respect to an outer, local niche. This would imply that some ongoing, mutator-driven phenotypic changes (across generations) could become ongoing, rapid evolutionary changes that are apparently adaptively neutral with respect to an outer, local niche, but are not so with respect to the consequences of the stressful, mutual imprinting within an inner niche, the genome. In effect, such evolutionary changes

would, for the most part, be an extension of an ongoing accommodation to the inner, genomic niche. Through such would come the enhancement of the inner-parameter of evolution. Moreover, some of those changes due to this inner-accommodation might also be related to changes in traits that are more obviously related to stresses of local niches.

As implied, among the consequences of mutual, dynamic imprinting, ensuing through stress, would be an adaptive, inner redirection of dynamical effects conveyed through changes in molecular structure and processes, and on the genomic level, through inner-directed, transmissible changes in organization. In this way, dynamic imprinting will have also become a conveyor of a type of information to progeny, providing still another inner-avenue ensuing in evolutionary change.

These biomolecular structures/processes would be, along with the membrane structures of the cell, the conveyors of the specific type of forces, e.g., electrical cohesive forces of given configurations and degrees, needed in or expressed through adaptive developmental reorganization or generation from the genomic level to the organismic level. In this light, the genome does become coextensive with the eukaryotic cell as Von Sternberg argues [1996], and, as he further points out, the cell can be involved in directing genomic self-modification. However, it is the cell as an environmental generator and conveyor of complex, non-uniform forces to itself and to the eukaryotic, nuclear genome, and the genome's and cell's particular, directed response due to such forces, that is the essential factor or "center piece" here.

In this regard, stressful, cohesive forces mediated by particular biomolecules and cellular structures may necessarily be involved in the adaptive development or generation of plantlets from plant tumors *in vitro* (Lieber [1996]). Most relevantly, development or generation of particular organ systems in amphibians and in certain mammals have been induced by or related to changes in externally applied electric fields at the regions where the regeneration was to occur (Becker and Selden [1985]; Becker [1991]). Moreover, in frogs the application of electric fields to bone fractures led to regeneration of bone through a dedifferentiation of cells that in turn redifferentiated. Such application was also found to have

greatly enhanced m-RNA and protein synthesis in the cells. Becker and Selden concluded that this dynamic, electrical effect must have been conveyed through the electrically charged membranes of the cells. Furthermore, they have shown that mechanical stresses or forces applied to bones generate electrical fields within bones leading to bone growth that consequently strengthens, and hence stabilizes, the bone to the mechanical stress field. Predictably, in this connection, force-fields could also stimulate immunological processes as developmental responses. In fact, "results show an overall *in vivo* immunopotentiation of humoral and cell-mediated immune response in rats exposed to [locally applied] magnetic fields" (Jankovic *et al.* [1991]). And, in the treatment of patients having endometrial cancer, it was found that EM radiation of shortwave frequency had an immunostimulating and immunomodulating effect in such patients (Zaporozhan *et al.* [1993]).

All of these regenerations and immunological stimulations may very well have been developmentally adaptive and generative responses to these stressful force fields intersecting with the organism at various levels, where the very force fields themselves, through the consequence of imprinting, could have provided the complementary, generated avenues necessary for these adaptive responses. Such developmentally adaptive responses, involving these fields, may very well have implicated an epigenetically controlled global mutagenesis. In many cases, development does involve genomic changes (e.g., Fischberg and Blacker [1961]). Development becomes a non-linear extension of genomic mutation, or perhaps genomic mutation can be seen as a non-linear extension of what has classically been considered development. As pointed out, during evolution, features of mutator systems could have become incorporated into development.

At its deepest and most inclusive feature, development becomes a continual, inner-directed reorganizational solution to repeated, stress-induced changes in organization on various levels. This reorganization necessarily generates and re-establishes, through dynamical complementation on all levels, an increasing global uniformity of force throughout the diversity of the organism. This is a reorganization that at the genomic level can be transmitted to progeny with fast, evolutionary benefits.

In this context, environmentally connected mutator processes, which control mutagenesis on a global basis and involve different levels of genomic organization, would themselves be epigenetic markers or manifestations of adaptive-conferring force fields. By means of and through such force-fields, giving the organism hydrodynamical-like properties, apparent, absolute boundaries between organism and a stressful, mutagenic environment would fall away or become re-defined in such a way where the organism would assume a greater functional integrity, and hence adaptiveness. It is an adaptiveness that is marked by the enhancement of the immunological self through completion.

What adaptation or accommodation ultimately comes to mean, in this light, is the generation, through dynamic completion, of more stable force configurations within the genome, between genome and the rest of the cell, and between the cells and their environment, including other cells. What is in fact a mutagenic stress to an organism, be it in the form of nutritional stress or an antigenic stress or an EM field, is ultimately posed to the organism as a non-uniform, and hence destabilizing, force configuration. This destabilization of force must be resolved through a developmental mutagenesis of the genome and a contingent organismic development. This resolution would be driven by that very dynamic instability into an adaptively, more uniform force configuration throughout the diversity of the genome, and ultimately via development and developed reproductive organs, throughout the organism and to subsequent progeny in a short period. The immunological response, and its molecular aspects, would be but a manifestation of such an underlying dynamics that can be extended through organisms and across generations, making the scale of evolution itself a generative solution to non-uniform force.

If mutator processes behave universally in a developmentally, adaptively responsive manner to dynamical imprinting, and this type of response is hence not an aberration, this should become more and more demonstrated in further experiments on diverse organisms and their progeny. Adaptively responsive mutagenesis to low levels or degrees of local stress ought to be found in general, and this type of mutagenesis would be through ultramutability systems.

The particular type of stress would not be important in such experiments. It could be of any type, such as EM fields or nutritional deprivation or stress mediated through classical chemical mutagens. It would be stress as a non-uniformity of force imprinting on, and hence intersecting with, the genome that would be important, as well as its degree. In these experiments, the consequence or resultant dynamics should be a co-ordinated, global ultramutability, adaptively responsive to the low level of stress in the form of non-uniform forces. As stated, underlying this mutagenic, adaptive response to stress would be, through dynamic completion, the generation of a greater uniform force configuration within the genome, and later via development, throughout the organism, which would later be present in progeny. The necessity of this generation would reflect the operation of a unifying principle.

The degree of local stress would have to be of low but proper level so as to mediate and allow the adaptive response. If the stress were to be severe, the process of mutagenic response would be impaired, and the generation of deleterious mutations could ensue. If, in contrast, the mutator system were subject to too little local stress, the generation of apparently non-adaptive, transmissible "neutral" mutations might very well occur. However, these latter might very well be attuned to other, more subtle, non-apparent stresses.

An important experimental prediction can thus be made. In general, there will be found a causal relationship between adaptively responsive mutagenesis and global genomic changes due to low degrees of dynamical imprinting upon the genome. After such adaptive mutagenesis, the existence of more coherent or uniform force fields throughout the genome should also be detected, especially in progeny. This might be marked by an increase in electromagnetic resonance within the genome. It is further predicted that this will become to be associated with new, genomic molecular markers in progeny or with changes in those markers already known.

This situation could have very important agricultural and medical applications. For example, many crop plants inheritably saline-tolerant could readily be isolated in short periods, via a tissue cul-

ture situation, using a very low level (or concentration) of a classic mutagen-stressor, such as nitrosoguanidine, in growth media also containing low levels of saline. In an analogous manner, numerous crops inheritably resistant to various toxins could easily be isolated, substituting in such cases the given toxin, in low level, for the saline situation. Perhaps, inheritably toxin-resistant farm animals could also be isolated using similar experimental approaches.

In earlier experiments, some years ago (P. Lieber and M. Lieber [1974]), *Drosophila* were exposed to very low levels of X-rays (0.5 to 3 roentgens) while on culture media containing low levels of the pesticide, DDT. Controls in which there were different patterns of exposure and non-exposure were also conducted. It was repeatedly shown that such exposure greatly increased their degree of survivability, in terms of increased population sizes, within two to three generations as compared to flies not exposed to both radiation and DDT, and as compared to flies exposed to DDT alone. On media not containing DDT, fly populations exposed to 3R radiation did not have any terminations, even after the 11th generation, compared to populations not exposed to radiation. These observations would certainly suggest that very low levels of mutagenic stress in the form of a high energy electromagnetic radiation, namely X-rays, can have beneficial adaptive effects, especially in synergy with other environmental stressors in low degree. It would be very important to repeat this type of experiment using other types of animals, especially with the aim of isolating other types of beneficially responsive mutator systems and their dynamics.

In the 1960s, it was predicted by P. Lieber that a beneficial radiation effect upon adaptive evolution would be widespread. Radiation in low degree was seen by P. Lieber as making the genome globally metastable so that it would be enabled to generate a global, adaptively responsive mutagenesis to internal and external stress. Though not addressing evolution *per se,* extensive research has, nevertheless, given ample support to this prediction. Very low levels of ionizing radiation does benefit animal growth and development, fecundity, neurological function, general health, and longetivity (Luckey [1982]). Also, recent results (Feinendegen *et al.* [1998]) have shown that very low levels of X-rays can have a

beneficial effect in higher mammals by acting as a cancer preventive. Moreover, low-dose X-ray irradiation enhances the ability in mice to regulate energy metabolism (Yamaoka [1997]). All of these observations may suggest that low level irradiation may be essential to life itself, and hence, significantly beneficial for evolution. The nature of radiation's interaction with life may thus be indicative of an underlying, unifying dynamics operating in the universe and so reflected in biological evolution as an inner-directed parameter.

7. ADAPTATION TO LOCAL AND GLOBAL NICHES IN EVOLUTION

As we have seen, global hypermutability or its mutator source reflects the inner-directed, adaptive response to the local and inner, changing stressful niches of organisms. It would have been a significant, increasingly inner dynamic in evolution. However, there appears to be a type of mutation that is responsive to a far more inclusive global or *universal niche,* suggesting that there is also a level of evolution responsive to such a universal niche. These mutations appear to mark, as a universal evolutionary clock, the constant rates of structural gene evolution described by Wilson *et al.* ([1974], [1977]). Such linear mutation appears not to have been affected by the stress of local environments, but would appear to have been attuned adaptively to the more uniform forces of a universal niche. Spontaneous mutation arising and detected under locally non-selection (non-stressful) conditions may be a consequence of mutagenic processes responsive to a universal niche.

According to a principle first described by the physicist Ernst Mach in the last part of the 19th century, the inertia (or inertial force) of any body or physical structure, no matter how small, is determined by the gravitational forces of all the other bodies in the universe. The constant rate of structural gene evolution may reflect a gene mutation arising through a constant, generative accommodation to a universal determination of inertial forces. The organism and its genome must hence simultaneously adapt to the

stresses of a local niche and to the more uniform, inertial force stress determined by the universal niche. The universal solution that would enable such simultaneous adaptation would be in the form of spiral generation of mutation. Mutation becomes a type of regenerative growth.

When one takes the ratio of the highest stress-related hypermutation rate involving a gene in *E. coli* (referred to in Lieber [1989]) to that of highest general, non-stressed related spontaneous rate involving a gene in *E. coli* referred to earlier, one obtains the dimensionless value 1.6×10^4. If two genetic regions or markers are jointly considered in this regard, the ratio becomes 1.6×10^{12}. 1.6, referred to a i/Q, is an important dimensionless constant of spiral generation that appears to necessarily pervade all of nature in an adaptive manner (Lieber [1998]). This would include the DNA helix itself in whose very spiral form a facet of the co-adaptive solution, referred to above, may geometrically be represented.

Calculation shows that the ratio of the angstrom distance along the DNA axis between any two of its cycles (or turns) to the angstrom diameter (width) of the DNA helix is constantly very close to the dimensionless constant 1.6. (34 angstroms/20 angstroms = 1.7). This constant ratio may also indirectly refer to or represent the largest, constant unit-degree of maximum hypermutability or dynamic instability that can be generatively displayed by a constant unit-section of DNA *in vivo*. This unit of maximum dynamical instability may represent a constant of regeneration within DNA that re-establishes DNA stability through a directed dynamic completion. This would be a generative completion connected to local and global forces and made necessary by them, and marked molecularly by a controlled misrepair. Such a spiral, generative completion would be defined by i/Q and be related to the intersection of non-uniform force with the critical sections of DNA. i/Q would also mark the boundaries of this generative intersection.

In the respective ratios of mutation rates in which i/Q became evident, the very large integers associated with i/Q each might also refer to the particular level of genetic organization responding generatively, and hence mutagenically, to local and global stress.

Such integers might also be markers of the degree of non-linear compounding of a unit-generative-mutagenic response marked by i/Q. This would occur through continual spiral generation at different genomic levels, including that of DNA, and, by virtue of such generation, the genome could become stabilized as a spiral, higher-order structure most accommodated to local and universal niches. The generative spiral implicitly becomes an evolutionary solution on every scale.

Wilson *et al.* ([1974], [1977]) have shown that morphological evolution in mammals and herbaceous plants is correlated with karyotypic evolution. Such morphological and karyotypic evolution has occurred at high, non-linear rates in contrast to the evolution of individual structural genes which has occurred at much lower, constant (linear) rates, and whose evolution is not correlated with that of morphological evolution. This may suggest that the higher the level of genomic organization e.g., the karyotype, the more responsive it is, in terms of adaptive change, to the changing, stressful conditions of local niches. This could have been manifested in the creation of new regulatory sensitivities of the genome to the environment. Wilson *et al.* have argued that changes at the karyotypic level could have created regulatory changes responsible for morphological evolution. Such regulatory changes may have created, in a helicyclical manner, new epigenetically responsive mutator systems that could have become normal features of development and passed on to progeny.

Cherry *et al.* [1978] do acknowledge that mutator processes operating at the karyotypic level may have contributed to bursts of very high rates of morphological evolution in certain vertebrate phylogenetic lines. Across modern day species, there has been great variation in degrees of mutation (Cherry *et al.* [1978]). This may have been due to the differential responses on the part of various mutator systems to the different stresses of local niches. The lack of mutators in some lines may also have contributed to this variation. Especially under temperature stress, the mutator systems in the fungus *Aspergillus* did generate changes at the chromosomal level which were clearly associated with sudden, inheritable and programmatic changes in *Aspergillus* morphology and colour pattern (Lieber [1972], [1976a], [1976b]). Strains of *Aspergillus* with-

out mutators did not respond in this manner to temperature stress.

It would thus seem that high levels of genomic organization are best enabled to respond most adaptively and non-linearly to local stresses, generating an evolution most attuned to such stress, while far lower levels of its organization are being accommodated or responsive to the universal niche in a constant or linear manner. Thereby, an evolution would also arise that is attuned to the type of stress of the universal niche. i/Q would define the universal, non-linear, spiral parameter through which these differentiated responses are enabled and reconciled, with respect to one another, and would point to a regenerative, universal principle of spiral completion operating in nature.

Mutagenicity on whatever level, including exceedingly responsive levels that even go beyond the genome, transgenomic, such as the organelle configurations of the cytoplasm, would be a feature of such a regenerative completion that brings an adaptive, cohesive uniformity of force, hence coherence, within the evolving designs of nature. More generally, there may be hierarchies of evolution in nature through which this is manifested (P. Lieber and M. Lieber [1983]). Through the mutagenic, generative parameter in such hierarchies, the dimensionless biological constant i/Q, and similar constants, become manifest.

8. CONCLUSION: DIRECTED MUTATION AND AN ORDERING PRINCIPLE

This article has presented ideas towards a unifying perspective on mutation. The present, mainstream explanations as to the origin of responsive hypermutability do not address the real significance or meaning of this phenomenon. This is perhaps due to limitations of the molecular paradigm through which explanations of this phenomenon are generally made. As long as such paradigms are only used, the deep, universal significance of directed hypermutability will not be grasped. This article has attempted to shed some light as to the deeper, universal source of such a phenomenon, and hence its true meaning.

The future demonstration of the universality of adaptively responsive phenomena to stress i.e., non-uniform force, and their basis, would point to the existence of a universal principle governing the behaviour of forces encompassing all levels of biological processes. Biology has long sought such a unifying principle, and this article has revealed or touched upon an aspect of such. A further illustration of such a principle was given by P. Lieber [1969] and M. Lieber [1996] and is further described by M. Lieber [1998] through its connection with a universal, dimensionless biological constant that pervades the universal physical constants.

Other imaginative, related roads toward elucidating a unifying principle have also been made. These relate to descriptions of coherence domains in physical and biological matter (Preparata [1992]; Del Giudice [1993]; Ho [1993]; Sermonti [1995]). Such coherence domains are seen to be based upon resonating electromagnetic force fields generating within the domains. Through such resonating fields, biochemical components and processes lose their individual identity and move and are brought together in a resonating, coherent, and ordered fashion so as to promote the function of the domain. It is the frequency of resonation which determines the given order. EM fields of different resonant frequency applied or imprinted upon the domain can change its inherent resonation, and hence change (or mutate) the pattern of order in such a way that the integrity of the coherence domain is maintained with possibly even greater coherence.

According to A.F. Popp ([1972], [1984], [1984/1985]), DNA itself generates EM fields of given resonating frequency necessary for its stabilization and that of the cell. The specific resonating frequency depends on DNA configuration. Evidence suggests that chemical mutagens or carcinogens in contact with DNA interfere with its stabilizing resonating frequency. They and the DNA configuration are seen as becoming altered so that a stabilizing, cohesive resonating frequency is re-established. If it is not re-established, neoplasia could ensue, according to Popp. With regard to what has earlier been said in this article, many of these features of coherence domains could be aspects of the adaptive pattern of internal, dynamic completion generated following the imprinting of dynamic non-uniformity. Future work could bring these related

approaches further together.

For now, one may have been persuaded of at least one conclusion: No matter the specific roads used to reach it or to describe it, one must come to see that a generative, hence dynamical, ordering principle is operating in the universe in which directed mutation is but one of its manifestations. This principle would enable the various, possibly hydrodynamical, features of the universe to accommodate one another, no matter the scale. In this context, randomness and entropy become limited cases at best.

In considering life, the physicist Erwin Schrödinger [1956] proposed that a principle of negative entropy (negentropy) was operating, a principle defining an ever increasing order, as opposed to increasing disorder as represented by increasing entropy. He did not believe such a principle was contrary to or beyond physics, but would require an imaginative extension of physics. He represented this negentropy by the following equation, the inverse of the classic entropy equation: -(entropy) = k log(1/D). D is the quantitative measure of the atomistic disorder of a body or situation; k is the Boltzmann constant (3.29×10^{-24} cal./°C.), and it in fact equals $2(i/Q) \times 10^{-24}$ cal./°C.

We hence see the re-appearance once again of the universal, dimensionless biological constant, that ever pervasive constant, in another yet similar guise. In this case, it marks the spiral generation of completing force through a parameter of irreversible time, by means of which, an evolving order, an increasing uniformity within non-uniformity, would occur. The irreversible time would be projected or incorporated into the evolving, local situation, but be endemic to the universal niche. The i component of this constant does define a constant, quantum-regenerative time (Lieber [1998]). This regenerative-time quantum would be a feature of a universal, unifying ordering principle that enables adaptation on all levels. This universal, ordering principle would imply an inherent determinism. A feature of this would be a spirally generative complementation or completion. Mutation, growth, and reproduction would be expressions of such a dynamical complementation, on all scales, which would continually give rise to symmetry in response to continual imprinting at foci of dynamic intersection. Ultimately, these expressions are necessary manifestations of

an ordering principle.

The perspective given by this principle would suggest a deeper, more extensive meaning of mutation and evolution, and one that could lead to new research breakthroughs. If the molecular approach to genetics and development were to interface in a complementary manner with a force-field approach or interpretation, both encompassing a universal principle of dynamic accommodation operating on all scales and orders, an avenue expressing these new perspectives on mutagenesis, new productive, research avenues and insights into immunology in particular, and biology in general, would surely ensue. The ultimate consequence would be the elucidation of a universal principle governing biological and physical phenomena and their generative connections through different levels, generative connections that are defined by a dimensionless biological constant.

"Things" are dynamically connected, hence ultimately cannot be random. The challenge for science is to elucidate the pattern and inner of such connections, to find "the go of it" as the physicist James Clerk Maxwell would have said. To do so requires us to transcend the limitations and incompleteness of our thinking, our own paradigms, whatever they are, to reach, to ever reach beyond arbitrary boundaries. With courage, this will be done.

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Michael M. Lieber

SISTEMI MUTATORI RISPONDENTI ALL'AMBIENTE: VERSO UNA PROSPETTIVA UNIFICANTE

Riassunto

La biologia ha cercato a lungo un principio unificante. Questo può intravedersi nel comportamento di processi mutativi geneticamente controllati, che rispondano adattativamente all'ambiente. Sistemi mutatori esistono in diversi organismi, dai batteri ai mammiferi. Tali sistemi sono emersi l'uno dall'altro e hanno definito la vera evoluzione degli organismi. Molti di questi sistemi mutatori possono determinare, nel corso dello sviluppo, una ultramutabilità o ipermutabilità estese a tutto il genoma. Benché il controllo genetico di alti livelli di mutabilità sia passibile di interpretazione molecolare, tali processi mutageni rimandano a un parametro più profondo che implica le forze e le loro configurazioni. Queste debbono stabilizzarsi a partire da stati instabili, attraverso tutto il genoma e l'organismo. La mutazione diretta diventa così un processo generativo in accordo con forze non uniformi di nicchie locali e con forze più uniformi di una nicchia universale. La modalità della risposta mutagena armonica dipende dal livello di organizzazione genomica e transgenomica. Questo è riflesso in gerarchie di evoluzione. La mutazione diretta è un aspetto di un processo generatore universale, contraddistinto da una costante adimensionale universale. È la conseguenza dinamica di tale generazione diretta che alla fine conferisce l'adattamento, attraverso il completamento dinamico. Ciò suggerisce una dinamica soggiacente, unitaria e necessaria che connette i sistemi di ultramutabilità in tutti gli organismi e potrebbe consentire, in complementazione con un approccio molecolare, di evocare nuove e produttive strade di ricerca. Queste potrebbero condurre a un principio unificatore, alla base dei fenomeni biologici e di quelli fisici.