Robert H. Haynes

Genetic Stability with Evolutionary Change

Abstract

In the absence of mechanisms to promote an extremely high level of replicational fidelity, the long genetic messages of contemporary organisms could not have evolved. In addition, were it not for the existence of processes which effect the repair or bypass of damage to DNA which arises from many ever-present natural sources, cellular activity would collapse from what might be called `genetic meltdown'. On the other hand, if these mechanisms were capable of functioning with perfect accuracy, genetic variation, and hence evolution by natural selection, would not have occurred. Thus, at the molecular level, the amazing diversity of life appears to be an adventitious consequence of the inability of natural selection to defeat thermodynamics and thereby extinguish itself by driving mutation rates to zero.

Introduction

Theodosius Dobzhansky often said that `in biology, nothing makes sense except in the light of evolution'. Unfortunately, the explanations that emerge from most traditional studies in evolutionary biology necessarily entail much conjecture and speculation regarding unrepeatable, highly contingent, events which took place in the far distant past (Dobzhansky, 1970). Normally, the best that can be achieved in such `historical' sciences is the construction of plausible stories which attempt to bring some degree of consistency and coherence to those observations which *can* be made in the present. It frequently transpires that more than one such story can be envisioned, and that one person's plausibility may be another's improbability. Thus, it is not surprising that, quite apart from puerile attacks by creationists and other connoisseurs of the irrational, evolutionary theory has been afflicted

with much controversy, and even rancorous debate, among the heirs and disciples of Darwin.

Like the erosion of the Grand Canyon, biological evolution is a continuing process today. In analogy with the principle of uniformitarianism in geology it is possible to gain some insight into the molecular basis of evolution by studying experimentally those biochemical processes responsible for the phenomena of genetic stability (heredity) and genetic change (mutation, recombination, etc.). In this paper I summarize some of the most general features of these processes at the macromolecular level and review speculations regarding their *apparent* adaptive significance as they have emerged from the discovery of DNA repair and its relation to mutation and recombination.

Natural selection is an immediate, or proximal, cause of evolutionary change, as observed at the phenotypic level. However, it is not a force of Nature analogous to gravity or electromagnetism. The *physical* sources of evolutionary change lie not in selection, but rather in the apparently random occurrence of heritable variation among organisms (Haynes, 1987). Indeed, Darwin himself emphasized that in the absence of variation, selection can do nothing.

Heredity, the fact that `like begets like' is a conservative process. It is a manifestation of the stability of the genetic material, and its accurate replication, transmission, and utilization from one generation of cells and organisms to the next. On the other hand, variation is a subversive process. It is a manifestation of many different sorts of change in the semantic content and structure of genomes. The molecular basis of genetic stability and change, and the surprisingly intimate relation between these superficially conflicting phenomena, is now known to be rooted in the chemical nature of the genetic material and the biochemical mechanisms for its replication, repair, recombination. Thus, I would extend Dobzhansky's aphorism and assert further that in evolution nothing makes sense except in light of the molecular mechanisms of genetic stability and change.

Genetic Stability and Change: Physics or Biochemistry?

In 1935 Max Delbrück suggested that the stability of genes could be explained physically in terms of the Polanyi-Wigner theory of molecular fluctuations (Timoféeff-Ressovsky et al., 1935). On this theory, spontaneous mutations were considered to arise from quantum-statistical

fluctuations in the genetic molecules. Mutations would occur rarely if these molecules were assumed to have unusually stable structures. Induced mutations also were thought to be purely physiochemical events arising immediately and directly from the interactions of radiations having high quantum energies with the genetic material, as envisioned in the classical target theory (Lea, 1946).

It is now known, however, that the genetic material is composed of ordinary molecular subunits and is not endowed with any peculiar kind of physicochemical stability. It is subject to many types of spontaneous structural degradation as would be expected in warm, aqueous, intracellular environments (Saul and Ames, 1986). In addition, cells are exposed naturally to many mutagenic agents of both endogenous and exogenous origin (Ames, 1983). Also, the potential error-rate of non-enzymatic DNA synthesis is high, the order of 10^{-2} per base pair replicated, whereas the observed error rates in normal replication are remarkably low, about 10^{-10} to 10^{-8} per base pair replicated (Reanny, 1987).

If the various sources of DNA structural decay, damage and replicational error had free rein, neither the informational integrity of DNA, nor cell viability, could be maintained. The well-regulated metabolism of living cells would collapse from what might be called `genetic meltdown'. That this does not occur arises from the fact that, arrayed against these many destabilizing pressures, there exists an amazing battery of coordinated biochemical processes that actively maintain genetic stability and viability throughout the cell cycle. These stabilizing devices can be divided into three main categories: (i) those that promote the observed high levels of replicational fidelity during normal, semiconservative DNA replication, for example, 3'-exonucleolytic proof-reading by DNA polymerases and methylation-instructed mismatch correction (Loeb and Kunkel, 1982); (ii) those that repair, or bypass, potentially lethal or mutagenic damage in DNA (Strauss, 1985); and (iii) those that chemically protect DNA by neutralizing or detoxifying mutagenic molecules of both endogenous and exogenous origin (Ames, 1983). In addition, recent studies have shown that appropriate *in vivo* alterations in the pools of deoxyribonucleotides can provoke the entire range of genetic effects normally associated with exposure of cells to physical and chemical mutagens (Kunz, 1982; MacPhee et al., 1988). Genetic loci known to control various modes of DNA repair in yeast have pleiotropic effects on cellular responses to deoxyribonucleotide pool imbalances (Kunz and Haynes, 1982). Similarly, studies with various DNA polymerases have shown that the fidelity of DNA synthesis *in vitro* depends on the relative concentrations of the deoxyribonucleotides in the reaction mixture (Das et al., 1985). It also has been found that certain mutator phenotypes arise from

genetic defects in enzymes required for pyrimidine nucleotide biosynthesis (Meuth, 1984). Thus, it would appear that proper regulation of deoxyribonucleotide pools is yet another process which contributes to the maintenance of cell viability and gene and chromosomal stability (Haynes, 1985).

On thermodynamic grounds, if none other, these remarkable error-correction and repair mechanisms cannot function with perfect accuracy. Even though many mutations are deleterious, natural selection cannot drive mutation rates to zero and thereby eliminate the continuing production of genetic variation. At the level of phenotypic evolution, the opposing processes of genetic stability and change emerge as complementary, rather than antagonistic, phenomena.

Adaptive Significance of Genetic Error Correction

Enzymic mechanisms for both `dark repair' and photoreactivation of damaged DNA, and the maintenance of the fidelity of DNA replication, have been found wherever they have been sought, in viral systems and from the simplest to the most complex organisms. Thus, it was argued many years ago that the possibilities for repair inherent in the informational redundancy of complementary base-pairing may account at least in part, for the ubiquity of double-stranded nuclei acid as the genetic material of contemporary cells (Hanawalt and Haynes, 1967). All of this suggests that processes of genetic stabilization are of fundamental importance for living systems and that they arose very early in evolution (perhaps, in primitive, relatively ineffective, form even before the appearance of cells). Their importance for contemporary organisms is well-attested by the extraordinary sensitivity to mutagens of cells deficient in DNA repair: the LD37 dose of germicidal ultraviolet light (UV) for yeast mutants deficient in all three major repair processes is that which corresponds to the formation of only 1 or 2 UV pyrimidine dimers per genome (Haynes and Kunz, 1981). It is very unlikely that organisms totally deficient in DNA repair could arise and flourish in nature.

The replicational and protein synthesizing machinery of the cell is a remarkable example of a highly reliable, dynamic system built from vulnerable and unreliable parts. Many different genetic loci are involved in the biochemical stabilization of the genetic material, both directly and indirectly. The actual number of such loci in any organism is not known, but it is likely to be rather large (Haynes and Kunz, 1981). In engineering de-

sign, it is well known that if very great fidelity is to be achieved with equipment of poor intrinsic precision, extensive checking and quality assurance procedures must be built into the system. For optimum economy, the energy cost of such procedures should be just sufficient to reduce the overall error-rate to a tolerable level (Dancoff and Quastler, 1953). This `principle of maximum error' is exemplified in the genetic machinery of cells. The evolution of long genetic messages has been made possible, in part, by the fact that they encode extensive instructions for their own correction. The energy cost clearly is not prohibitive and the residual error-rate ist consistent with the genetic integrity of normal organisms and most of their progeny. Natural selection appears to have fashioned all major aspects of DNA metabolism to counteract the deleterious effects of `genetic noise' and thereby to minimize mortality and mutability. I call this the `3M' principle of metabolic design. However, in view of the existence of `error-prone' processes which simultaneously promote viability and generate mutations, it seems that viability takes precedence over genetic fidelity in the economy of cells (Witkin, 1969).

Discovery of the close relation between DNA repair and recombination has had a profound impact on current evolutionary theory (Howard-Flanders and Boyce, 1966). It has been argued that the adaptive significance of recombination (which appears to be phylogenetically ubiquitous) lies primarily in its ability to provide a mechanism for repairing damaged DNA, rather than the production of genetic variation in the form of new combinations of alleles (Maynard Smith, 1978). Thus, it is plausible to imagine that the two fundamental features of sex, recombination and outcrossing, are adaptive responses to DNA damage: recombination as a mode of DNA repair and outcrossing as a mechanism for `masking' deleterious recessive mutations in heterozygotes (Bernstein et al., 1988). If recombinational repair is more effective in germ-line than in somatic cells, then it is not surprising that the former should appear to be `immortal' while the latter should `age' as a result of the accumulation of DNA damage and mutations over the life-time of the individual (Medvedev, 1981).

The essential raw materials which make evolution possible, mutational and recombinational variation, may be merely adventitious by-products of processes selected initially to maintain genetic fidelity, cell viability and to reduce the generally deleterious effects of genetic noise. Similarly, it seems reasonable to think that the primary benefit of the inducible SOS response in *E. coli,* like that of the damage-inducible responses to alkylation and oxidation, is to ameliorate the toxic effects of the inducing agents, rather than to expand genetic variation (Demple, 1987). Nonetheless, it also it possible, in bacteria at least, that should appropriate new

mutations be produced by induction of the SOS response these could serve to reduce the probability of population extinction in noxious environments (Echols, 1982). However, it seems unlikely that sexually-reproducing organisms would increase their general mutation rates as an adaptive strategy: such a manoeuvre, in all probability, would weaken, if not extinguish, the species (Crow and Denniston, 1985). The various arguments that have been brought to bear against group selection in evolution also can be applied to theories which suggest that the primary adaptive significance of mutation and recombination is to provide genetic variation upon which natural selection can act in the future (Williams, 1975). It is wise to remember that molecules are stupid, they do not think ahead.

The adaptive significance of mismatch repair in maintaining replication fidelity in bacteria is well-established (Modrich, 1987). Recently, however, Radman (1989) has shown that the requirement for DNA sequence homology in general recombination is greatly relaxed in bacterial mutants deficient in mismatch repair. He has found that in mutants defective in long-patch mismatch repair (LPMR) intergeneric recombination occurs efficiently between *E. Coli* and *Salmonella typhimurium* which are about 20% divergent in DNA sequence. It would thus appear that LPMR is anti-recombinagenic, whereas very short-patch mismatch repair is hyperrecombinagenic. Radman (1989) suggests that mismatch-stimulated anti-recombination by LPMR acts as a general `proof-reading' system assuring fidelity of homologous DNA recombination and thereby plays an important role in chromosomal stability. A corollary of this proposal is that LPMR also may play a fundamental role in speciation by ensuring the sterility of interspecific crosses.

Conclusions

Thirty-five years ago, it was widely believed that the genetic material was intrinsically very stable and that it stood `isolated' in some fundamental way from the routine metabolism of the cell. Any suggested process which entailed the breakdown and resynthesis of segments of chromosomal DNA ran counter to an established orthodoxy. Indeed, this presumed metabolic languor was taken as evidence for the genetic role of DNA. The discovery of excision repair played a seminal role in the formulation of our current picture of the mechnisms of genetic stability and change, a picture based more on biochemical dynamics than molecular statics. Elucidation of the mechanisms of DNA repair and replicational fidelity has had an amazingly far—reaching impact on biology and medi-

cine. New insights into problems ranging from the etiology of genetic disease, aging, and cancer, to the origin and evolution of life, have arisen directly from work in this area.

Today, perhaps the most exciting and informative work in this field is based on the application of DNA sequencing techniques to problems of mutagen specificity and the identification and phylogeny of the genes and proteins involved in DNA repair and allied phenomena. It is possible that such data will enable us to reject, or have greater confidence in, current speculations regarding the adaptive significance of the mechanisms of DNA repair and mutagenesis. The accumulation of DNA sequence data also should provide us with some insight into the old problem of the relative importance of point mutations and chromosomal rearrangements in particular lines of evolutionary descent.

The discovery of error-free DNA repair, which may be regarded as a `homeostatic' control system, also brings into sharp focus one of the deepest unsolved problems of evolutionary theory: the origin of such regulatory systems at the molecular level (Pattee, 1973). We simply have no idea how to account for the spontaneous origin of molecular control mechanisms in terms of physics and chemistry. Thus, we do not know how primitive replication systems, with very limited coding capacities, can expand sufficiently to encode the additional information necessary to allow the evolution of long genomes. Until this problem is solved, we can claim only a superficial understanding of the physicochemical origin of the `metastable', ever evolving genomes of contemporary organisms.

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