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SHORT TAKE

# Epigenetic gambling and epigenetic drift as an antagonistic pleiotropic mechanism of aging

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## Summary

**Generations of biogerontologists have been puzzled by the marked intraspecific variations in lifespan of their experimental model organisms despite all efforts to control both genotype and environment. The most cogent example comes from life table studies of wild-type *Caenorhabditis elegans* when grown in suspension cultures using axenic media. While nuclear and mitochondrial somatic mutations and 'thermodynamic noise' likely contribute to such lifespan variegations, I raise an additional hypothetical mechanism, one that may have evolved as a mechanism of phenotypic variation which could have preceded the evolution of meiotic recombination. I suggest that random changes in cellular gene expression (cellular epigenetic gambling or bet hedging) evolved as an adaptive mechanism to ensure survival of members of a group in the face of unpredictable environmental challenges. Once activated, it could lead to progressive epigenetic variegation (epigenetic drift) amongst all members of the group. Thus, while particular patterns of gene expression would be adaptive for a subset of reproductive individuals within a population early in life, once initiated, I predict that continued epigenetic drift will result in variable onsets and patterns of pathophysiology – perhaps yet another example of antagonistic pleiotropic gene action in the genesis of senescent phenotypes. The weakness of this hypothesis is that we do not currently have a plausible molecular mechanism for the putative genetic 'randomizer' of epigenetic expression, particularly one whose 'setting' may be responsive to the ecology in which a given species evolves. I offer experimental approaches, however, to search for the elusive epigenetic gambler(s).**

**Key words: antagonistic pleiotropy; bet-hedging; cell selection; epigenetic drift; epigenetic gambling; epigenetics; evolutionary biology; stochastic mechanisms.**

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Thanks to the generosity of the Keck Foundation, the National Academies, and the leadership of John R. Rowe, the 2007 Keck Futures Initiative meeting had as its theme, 'The Future of Human Healthspan: Demography, Evolution, Medicine, and Bioengineering' (for summaries, see http://books.nap.edu/catalog.php?record_id=12084). Among the many interesting lines of discussion was the counterpoint between the relative value of research attempting to understand the basis of the apparently stochastic variations in lifespan (and its coupling to healthspan) that play out among individuals within a species vs. research attempting to understand the basis for the more striking variations in lifespan (and healthspan) that occur when one compares different species. Given that the focus of that meeting was upon variations in healthspan within our own species, I had championed the importance of research on the former. Given the fact that an entire book has already been written on stochastic variations in lifespan by two leading biogerontologists (Finch & Kirkwood, 2000), I was surprised that my colleagues were generally dismissive of that approach. By contrast, they were highly enthusiastic about comparative gerontology. While I share that enthusiasm, the two lines of research are certainly not mutually exclusive. Because of the continued comparative lack of attention to stochastic mechanisms underlying intra-specific variations, however, I thought it useful to develop this essay in hopes that more can be done in that direction.

In preparation for those meetings, which took place at the Arnold and Mabel Beckman Center at the Irvine campus of the University of California Irvine, I created a preliminary document (with the able assistance of Judy Campisi) for a task force on 'Cellular and Molecular Mechanisms of Biological Aging: The Roles of Nature, Nurture, and Chance in the Maintenance of Human Healthspan'. The premise of that document was the evidence for a dominating role of stochastic events in the determinations of intraspecific variations in healthspan and lifespan. The best evidence for this hypothesis comes from life table data for the N2 wild-type strain of *C. elegans.* As these nematodes are hermaphrodites, every diploid locus is driven to homozygosity – i.e. they are comparable to identical twins. Thanks to the pioneering research by Vanfleteren *et al.* (1998) satisfactory life table parameters can be developed when these organisms are grown in suspension cultures using axenic media. In those experiments, one observes what experimental biogerontologists have observed for many decades – namely that, despite all efforts to control genotype and environment, there are very substantial variations in the lifespan of individual organisms. For these experiments with *C. elegans*, it is hard to imagine better controls of the contributions from Nature and Nurture. For such creatures, at least, the conclusion seems to be inescapable that





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chance events are the dominant contributors to the observed distributions of various degrees of what might be referred to as 'unsuccessful', 'successful' and 'elite' aging (the extremely long-lived outliers). This is in contrast to the situation for interspecific contributions to lifespan and healthspan, in which contributions from the constitutional genome dominate.

This essay elaborates upon stochastic contributions to intraspecific variations in lifespan and healthspan and takes, as its point of departure, a novel evolutionary hypothesis. I propose that these variations in 'successful' and 'unsuccessful' aging are a price we pay (or a prize we win) for a mechanism that evolved to ensure reproductive success of populations of young organisms facing diverse and dangerous environmental challenges. I use the term 'win' to refer to cells with enhanced function within an organism as well as to the entire organism containing those cells, which should also enjoy enhanced function and, thus, better prospects for reproductive fitness as compared to its siblings. I propose that there is a yet-to-be-defined plastic molecular mechanism(s) for 'setting' a degree of epigenetic variation in gene expression that is optimized for the ecology in which the species has evolved. I consider such 'epigenetic gambling' [or, to incorporate a more time-honored evolutionary idea, 'epigenetic bet hedging'; for a recent relevant paper, see (Crean & Marshall, 2009)] as intrinsic properties of the biology of all organisms, ranging from microbial species to mammals. These properties may well have evolved before the invention of meiotic recombination as a mechanism to provide sufficient phenotypic variation to ensure the survival of the species. Consider, for example, a group of organisms suddenly exposed to toxic amounts of a heavy metal such as cadmium. Genetic polymorphic variations, copy number variants and rare regulatory mutations in the gene actions that result in elevated steady state levels of various metallothioneins, gene products that chelate heavy metals (Nordberg, 1998) and thus protect against toxicity, will account for some differences in degrees of toxicity. One can imagine, however, an additional level of protection based upon random epigenetic variations at transcriptional and post-transcriptional levels such that some organisms would be more likely to survive and reproduce. What I am suggesting is a stochastically-based, environmentally-driven functional selection *within isogenic populations of cells.* The idea is pertinent to the enhanced functioning and/or enhanced survival of cells, their tissues, their organs and, therefore, the organism, including multicellular organisms like *C. elegans* and mammals, where the variegation can be assumed to be initiated during the development of families of differentiated cells. Consider, for example of how the liver responds to an environmental hepatotoxin. There is widespread but nonuniform cell death with subsequent regeneration *from multiple foci* of surviving liver cells, resulting in the classical anatomic pathological picture of a cirrhotic liver. I suggest that those foci are made possible by epigenetic gambling. Other possible examples of the variable susceptibility of isogenic families of differentiated cells could be cited, including responses of nigrostriatal dopaminergic neurons to mitochondrial toxins such as rotenone, of CA1 hippocampal cells to domoic acid or other excitotoxins, and of myocardial cells to viral or heavy metal-induced cardiomyopathies. Wound healing may be yet another example; the replicating fibroblasts of a healing wound are at various stages of the cell cycle (as they are when grown in culture), a clear advantage if the wound is contaminated with an agent that has specificity for cells within the G1, S, G2 or M stages of the cell cycle. Recent research on mammalian apoptosis supports these ideas (Spencer *et al.*, 2009). In a commentary on that paper by Bastiaens (2009), he asks the question: 'Why do cells of the same population respond differently to external death-inducing stimuli?' His answer is that 'Individuality seems to originate from non-genetic differences in the levels and activation states of proteins.'

An entirely different category of epigenetic modulation of gene expression may also contribute to phenotypic plasticity, adding to the many mechanisms that have evolved to ensure survival of cells, tissues, organs and organisms under differing environmental conditions. I refer to the fascinating phenomenon of the buffering of allelic variants by chaperones such as Hsp90 (Rutherford & Lindquist, 1998).

Another stochastic epigenetic mechanism that has been demonstrated to result in mosaic variegation of gene expression in human subjects is monoallelic expression of autosomal loci; it may involve more than 1000 human genes, including the beta amyloid precursor protein involved in the pathogenesis of dementias of the Alzheimer type (Gimelbrant *et al.*, 2007).This too might have evolved to enhance phenotypic plasticity.

I imagine that, once the epigenetic gambling has been initiated, it could 'have a life of its own', there being no selective pressure, after the peak of reproduction, for nature to select against the propagation of such risky behavior. Thus, while particular patterns of gene expression would be adaptive for a subset of reproductive individuals within a population early in life, once initiated, I predict that epigenetic drifts will enlarge phenotypic variegation within all members of the population to a point at which it will be uniformly deleterious, resulting in variable onsets and patterns of pathophysiology and senescent phenotypes. If my colleagues will accept this line of thinking, this would then constitute yet another example of Williams (1957) antagonistic pleiotropic theory of aging. Such a scenario would be consistent with what has been described as the 'epigenetic drift' observed in aging monozygotic twins (Fraga *et al.*, 2005). The authors of that study concluded that this epigenetic drift was the result of variable environmental influences. While it is highly likely that environment can indeed contribute to such drift, I have suggested that epigenetic drift might result from random variations in gene expression (Martin, 2005, 2007a,b, 2009). That epigenetic drift does indeed occur among apparently identical terminally differentiated mammalian cell types during the course of biological aging has received important support from transcriptional assays in cardiomyocytes from aging mice (Bahar *et al.*, 2006). It is of course also possible that a particular pattern of epigenetic gambling might *enhance* structure and function in the context of an aging physiology; I would imagine that this would be an unusual event, however.

A much more likely outcome would be biochemical oscillations that eventually go beyond some window of homeostasis (either excessively narrow or excessively wide excursions), a mechanism of aging suggested in a recent summary by Yates (2008).

There is a large microbiological and theoretical literature on a form of epigenetic variegation associated with transcriptional and translational bursts (see, e.g. Dobrzynski & Bruggeman, 2009). Transcriptional bursts also occur in mammalian cells and can differentially impact gene expression between the two daughter cells from a single mitotic event (Raj *et al.*, 2006). A particularly compelling and very early hypothesis to explain stochastic variations in gene expression pointed to statistical variations in concentrations of molecules that are inherently found in very small numbers in any given cell (Spudich & Koshland, 1976). This would be the case for many transcription factors. These various studies are typically interpreted as being the result of 'thermodynamic noise'. Thermodynamic noise remains a viable explanation for stochastic variations in gene expression; that mechanism and the hypothesis of epigenetic noise are of course not mutually exclusive. In any case, none of the published discussions on thermodynamic noise have proposed that the generation of such diversity in gene expression is under natural selection and is therefore molded by underlying genetic variation. We have to look to the extensive literature on bet hedging to seek deeper evolutionary understanding.

Not all stochastic events, of course, can be attributable to epigenetic variations in gene expression. Somatic mutations, involving both the nuclear and mitochondrial genomes, surely are among the chance events that make for differences in patterns of biological aging and late life diseases. Nuclear and genome copy number variations within families of somatic cells may be surprisingly common in *C. elegans*, for example (Golden *et al.*, 2007). For mammalian species, mutational loss of tumor suppressor genes and activation of oncogenes are major players in carcinogenesis. The likelihood that a critical number of such mutations will lead to invasive cancers may be related to the emergence of mutations that greatly increase the chance of subsequent mutations (Loeb *et al.*, 2008). Two identical twins may have the same flux of somatic mutations, but the lucky twin may have a disproportionate number of 'hits' in pseudogenes, whilst the unlucky one may develop a mutator phenotype early in the evolution of the neoplasm. That said, there is also clear evidence for a role for epigenetic alterations in the pathogenesis of cancer (Feinberg *et al.*, 2006).

The key to validating the evolutionary hypothesis proposed in this essay would come from efforts to demonstrate such genetic control. One potentially informative experimental approach would be to mutagenize a clonal isolate of somatic cells in a search for mutations that resulted in either extreme attenuations or extreme exacerbations of cell-to-cell variations in gene expression. Given the fact that transcriptional variations can be buffered by proteins with relatively long lifespan, assessments of such variations would best be done at the level of proteins rather than at the level of transcription. Recent improvements in quantitative immunocytochemistry would likely permit a suitable degree of quantitation of such variability within a large cohort of cells. An ideal wild-type cell would be a mouse embryonal stem cell line or, perhaps, an induced pleuripotent stem cell. These pleuripotent cells can be differentiated into a variety of terminally differentiated cell types. One would have to develop methods, however, to eliminate microheterogeneity within such progeny. For example, cardiomyocytes derived from such pleuripotent cell lines, while likely dominated by those with phenotypic characteristics of ventricular cardiomyocytes, may also contain cells with properties of pacemaker cells and atrial cardiomyocytes.

There would be two major advantages of employing such pleuripotent cell lines. First, one could, in principle, observe the phenotypic consequences of the putative regulators of epigenetic diversity by making mice via germ line transmission from a chimera. Second, one would have the potential to map and clone the mutant locus. The key to the success of such a program of research would be the application of an efficient selective methodology. One such approach might combine selection for very *low* expression of an enzyme required for the activation of a cytotoxin concurrently with selection for very *high* expression of an enzyme that directly protects against a toxin. Brute force approaches, however, if properly automated, might be considered and might utilize the large-scale gene trap resource being developed at the Texas A&M Institute for Genomic Medicine. Thousands of tagged mutant loci are available in C57BL/6N embryonal stem cells (Hansen *et al.*, 2008). More realistically, early progress testing this hypothesis is likely to come from microbial and *C. elegans* geneticists. Perhaps an approach utilizing an RNAi type screen for limited variability in multiple nematode traits would be productive. The laboratory of Tom Johnson has already made important inroads via experiments that have led to the conclusion that the variations in life span seen in these nematodes are likely to have only modest heritabilities (Rea *et al.*, 2005) (TE Johnson, personal communication), a result that is consistent with the hypothesis developed in this essay. Unpublished work from the Johnson lab also indicates an increasing variance of expression of an apparently stable transgene during nematode aging (TE Johnson, personal communication); this is also consistent with the hypothesis.

New opportunities for a genetic analysis also can come from the fascinating observations on the striking epigenetic variegations in the marbled crayfish (Vogt *et al.*, 2008). The hypothesis also predicts a greater degree of epigenetic gambling in asexual species or in asexual phases of species, such as monogonont rotifers, that can switch life cycles from parthenogenesis to sexual reproduction (Serra *et al.*, 2008). A more straightforward initial prediction of the hypothesis, however, would be the demonstration of anticipated differences in cellular epigenetic variegation of gene expression within the tissues of strains of organisms that have either naturally or experimentally experienced marked differences in their ecologies over many generations. Strains that have been exposed to highly varied environments might be expected to demonstrate much greater

coefficients of variation, in comparable cell types, than those of strains exposed to rather boring, predictable environments.

If this hypothesis receives further support and the molecular mechanisms underlying epigenetic drift become fully understood, it is not inconceivable that interventions can be designed to moderate epigenetic drift and thus ameliorate its contributions to senescent phenotypes. Even if such translational research proves problematic, however, basic research in this area is of obvious importance to a fuller understanding of the mechanisms of phenotypic plasticity in the context of evolutionary biology.

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