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Hereditary stability and variation in evolution and development

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SUMMARY Evolution and development are both lineage processes but are often conceptualized as occurring by different and mutually exclusive mechanisms. It is conventionally asserted that evolution occurs via the random generation of diversity and the subsequent survival of those that pass selection. On the other hand, development is too often presented as proceeding via the unfolding of a deterministic program encoded in the DNA sequence. In biology, universal generalizations are rare and dogmas are often wrong for particular cases. Deterministic mechanisms contribute some of the new DNA sequences that subsequently become substrates for natural selection. Conversely, stochastic and selective mechanisms are intrinsic to development, and also to maintenance of the immune, and possibly, nervous systems. Cancer appears to be another process that straddles distinctions between evolutionary and developmental modes of hereditary change and stabilization. DNA sequence changes are an essential feature of many cancers, but there are also aspects of the disease similar to developmental lineage gone awry. The literature suggests that the cellular changes that give rise to cancer occur by mechanisms commonly associated with both evolutionary and developmental lineage pathways.

INTRODUCTION

Biological heredity occurs in two contexts: evolution and development. In either case, the qualities of heredity are stability and variation. Stability in heredity between generations is essential for viable offspring within a species. In development, stability of differentiated cell lineages is the basis for tissue formation. In evolution, hereditary variation gives rise to phylogenetic trees (i.e., the relationship of species through common ancestors). In development, hereditary variation gives rise to differing cell lineages that together shape an organism (Maynard-Smith and Szathmary 1995).

The mechanisms by which hereditary variations are generated and maintained tend to differ in evolution and development. Evolution entails changes in nucleotide sequence to achieve both the generation of variation and to insure its maintenance. In contrast, differentiated lineages of cells within an organism typically have the same nucleotide sequence in their genome. In developmental cell lineages, hereditary variants are generated by positive feed-forward such that one state leads to another, for example by cascades of transcription factors while hereditary stabilization occurs via negative feedback (Brenner et al. 1990).

Development and evolution are often interpreted to differ in strategy as well as mechanism. Development tends to be interpreted as the unfolding of a "developmental program" that is executed during embyrogenesis. The developmental program is an efficient way to reach a constrained endpoint. Evolution, in contrast, is often conceptualized in terms of stochastic generation of variation followed by natural selection. The evolutionary strategy is thought to be less efficient but more "creative" and able to fill all available phenotypic space.

Biology has itself developed, or evolved, with something of an "iron curtain" between its sub-disciplines of development and evolution. But molecular mechanisms or strategies that have been narrowly sequestered to one camp are often useful in the other (Thaler 1996). When examined carefully, many systems of biological lineage are not "pure" random sequence variation or "pure" expression stabilization. Understanding certain systems is aided by simultaneously considering them as both developmental and evolutionary. Two cases are considered in this review: (1) physiological states that increase and alter the spectrum of DNA sequence mutation; and (2) the somatic immune system of mammals. More speculatively, this review argues that cancer will be best understood as a system that simultaneously uses both evolutionary and developmental modes of lineage.

DEVELOPMENTAL AND PHYSIOLOGICAL THEMES IN EVOLUTION

Deterministic mechanisms associated with development generate some of the new genomic nucleotide sequences that are grist for the mill of natural selection. Barbara McClintock considered this intersection of development and evolu-

tion when she raised the possibility of "inducible evolution." "A goal for the future would be to determine the extent of knowledge the cell has of itself and how it utilizes this knowledge in a 'thoughtful' manner when challenged" (McClintock 1983). Bacterial systems have been especially important in providing evidence that mutation rate and type are heavily influenced by physiology.

The SOS system in *Escherichia coli* is the "type case" for a stress-inducible mutagenic response for which "inducible evolution" has been proposed (Echols 1981; Radman 1975; Witkin 1976). The bacterial cell senses direct and indirect indications of damage to its DNA (actually any DNA in its cytoplasm that contains certain signals). Mechanistically, damaged bases and stalled replication forks are bound by RecA protein and this RecA becomes activated as a nuclease specific for several targets including the LexA repressor. Among the approximately 20 genes that are under LexA control are *umu*C and *umu*D. *umu*D is further processed by activated RecA and together these three proteins facilitate mutagenic synthesis (Ohta et al. 1999). This mutagenic synthesis is immediately beneficial to cell survival because it allows synthesis over sites that lack normal base-pairing information. These sites otherwise block progression of DNA replication and prevent cell multiplication. Enhanced mutagenesis is also "inducible" and "useful" because it is invoked in response to the suffering of life-threatening stress. Enhanced mutagenesis occurs at undamaged as well as lesioned sites in SOS-activated cells. The mis-insertion bias as well as the rate are different from the background rates in physiologically normal cells (Yatagai et al. 1991) (i.e., the spectrum as well as the amplitude of mutation is altered).

Nutritional starvation also induces stress responses that engender genetic change including the activation of transposition (Hall 1988; Maenhaut-Michel and Shapiro 1994; Shapiro and Higgins 1989) and point mutations (Cairns et al. 1988; Foster 1998; Hall 1990). From the standpoint of molecular mechanisms it is conceivable that the mutagenic effects associated with a cell sensing its environment and history could be as exquisitely regulated as transcription often is (Davis 1989), perhaps through the action of the same factors (Liu and Doetsch 1998). Transcription plus high levels of guanosine tetraphosphate in *E. coli* is reported to increase mutagenesis specifically in genes are that are ppGpp induced (Wright 1996, 1997; Wright and Minnick 1997). The extent of focused mutagenesis during stress responses is controversial with critiques and counter-critiques of experimental design and interpretation. Distinguishing a specific from a generalized stress response has sometimes been confounded because of selective outgrowth under the non-lethal regimes needed to assess stress-induced mutation (Andersson et al. 1998; Lenski and Mittler 1992; Thaler and Messmer 1996). The reversion of a particular *lacZ* frameshift during selection for lactose utilization (Cairns et al. 1988) was originally proposed to be "adaptive" (i.e., the mutation's probability of occurrence is greater when it would be of benefit to the cell). This system has been reinterpreted and reversion of the *lacZ* frameshift allele appears to be part of a more generalized stress response (Foster 1997; Torkelson et al. 1997), or perhaps no stress response at all (Andersson et al. 1998).

Specialized states for generating generalized variation occur in both prokaryotes and eukaryotes. Transformation competence in bacteria and meiosis in eukaryotes are both differentiated states for generating genetic variation via homologous recombination, and both are bona fide developmental processes. In the well-studied cases of *Bacillus subtilis* and *Sacharomyces cerevisiae* the induction of competence (Hamoen et al. 1998) and meiosis (Chu et al. 1998), respectively, are triggered by nutritional exhaustion and/or cell crowding. To a first approximation, homologous recombination is equally probable anywhere along DNA sequences that are similar (first formulated by JBS Haldane). On closer examination, this approximation breaks down in two ways. Certain sequences encode cis-acting sites that elevate the frequency of generalized recombination in their neighborhood while other sequences are "cold spots" that depress nearby genetic exchange (Stahl 1979). Secondly, the degree of sequence similarity required for homologous recombination is itself under genetic control (Rayssiguier et al. 1989) and along with restriction-modification systems it is also physiologically modulated via pathways of stress response (Matic et al. 1995). Physiological modulation via the SOS system, and possibly other routes, breaks down the barriers to inter-strain, interspecies, and intra-chromosomal recombination.

Another class of short-lived mutators are not directly based on physiological modulation, nor are they based on genomic sequence changes in genes of genomic metabolism. This class of transient mutators is proposed to arise from stochastic errors in transcription or translation of DNA polymerase or DNA repair enzymes (Ninio 1991). The mutator phenotype would last only as long as the mutant protein, but the mutations it procreated become permanent in the genome. By the time mutant cells are selected and grown up there would remain no trace of the transient mutator state that gave rise to them. Transient mutators of this type are proposed to be the basis of "adaptive" mutagenesis in *E. coli* (Rosenberg et al. 1998).

It is important to distinguish between the two types of transient mutators that have been discussed. One is based on a stochastic mis-translation or mis-transcription of genes of DNA metabolism. The other is based on a specific response of the cell to the environment (including internal environment) as it senses it. There is the possibility of overlap between these mechanisms but such overlap has not been demonstrated. For example, under conditions of nutritional deprivation transcription or translation errors might increase

and thereby engender more frequent mutator transcripts or translates of DNA polymerase.

A stochastic approach followed by selection is the normal paradigm within which biological evolution is interpreted. When stochastic variation is applied to genes that themselves modify the quantity and type of variation generated, the consequences become much richer (Field et al. 1999; Magnasco and Thaler 1996; Taddei et al. 1997). Modeling and mathematical studies show that the potential to mutate and inherit mutator genes results in being able to escape trapping in local optima. This theory of the consequences of inheriting mutator alleles is grounded in biological fact. The inheritance and mutability of genes whose products affect the mutation rate over a large range (approximately 4 log units) is indisputable (Fijalkowska and Schaaper 1996).

Some mutators are cis-acting (i.e., they promote mutation in and around themselves but not at distant locations). Short repeated sequences in bacteria (Field et al. 1999; Moxon and Thaler 1997) and in eukaryotes allow the focusing of both frameshift mutations and of quantitative variation (King 1994; Trifonov 1989). Mechanistically, repeated sequences are frequent sites for the slippage of DNA polymerase (Streisinger and Owen 1985). Whole genome analysis of pathogenic bacteria led to the discovery of repeats in genes that interact with the host immune system. Cis-acting mutators in genes encoding surface markers allow the pathogen to combinatorially alter its presentation to the host's immune system (Moxon and Wills 1999). Interactions between cis- and trans-acting mutators are to be expected, and they occur due to the recognition of particular sequences by enzymes that catalyze rate-limiting steps of recombination or mutation (Thaler et al. 1988; Thaler and Stahl 1988).

Lamarckian explanations are largely discredited in biology. However, the categorical rejection of models with any hint of Lamarckian elements exceeds molecular and physiological necessity (Clark et al. 1993; Landman 1991). Molecular mechanisms consistent with biology and physics have been proposed for the inheritance of acquired characteristics (e.g., Cairns et al. 1988; Kang and Temin 1973; Stahl 1988). Gametes of plants derive from cell lineages that have been subjected to environmental selection. Weissman's doctrine of germ cell lines being absolutely segregated from somatic tissue—and thereby somatic selection—may not apply to many animal phyla (Buss 1983).

The induction and focusing of mutation and recombination implies that evolution uses some of the same DNA metabolism mechanisms that are utilized in a (perhaps) more orderly manner in development. That does not mean that evolution should be understood deterministically, but it suggests that stochastic processes can be harnessed either toward predictable outcomes or toward open-ended processes. In the words of Lewis Thomas, "The capacity to blunder slightly is the real marvel of DNA. Without this special attribute we would still be anaerobic bacteria and there would be no music" (Thomas 1990).

EVOLUTIONARY THEMES IN DEVELOPMENT

There is a complementary literature in which either or both of the two pillars of evolutionary explanation—a stochastic generation of diversity and subsequent selection by sieving—are applied to developmental systems. Turing's chemical model of morphogenesis (Turing 1952) invokes small perturbations, including Brownian motion, to break symmetry assumed to be present during the initial states of embryogenesis. "It was assumed that the deviations from spherical symmetry in the blastula could be ignored because it makes no particular difference what form of asymmetry there is. It is, however, important that there are some deviations, for the system may reach a state of instability in which these irregularities, or certain components of them, tend to grow. If this happens a new and stable equilibrium is usually reached, with the symmetry entirely gone. The variety of such new equilibria will normally not be so great as the variety of irregularities giving rise to them. In the case, for instance, of the gastrulating sphere, discussed at the end of this paper, the direction of the axis of the gastrula can vary, but nothing else" (Turing 1952). Turing's model does not involve sieving or selection among individual cells or cell lineages. His model is equivalent whether the blastula is considered to be composed of a continuous field or of discreet cells.

A prototype success for models that use both stochastic and sieving processes in development is the clonal selection theory of immunology that prevailed over the instructionalist model championed by Linus Pauling (Podolsky and Tauber 1998; Tonegawa 1983). Selectionist models have been proposed (Changeux et al. 1973; Changeux and Danchin 1976; Edelman 1993)—but not proven (Crick 1989; van Belle 1997)—in the context of neurobiology regarding the strengthening of synapses and survival of neurons. Neural net simulations for both the nervous and immune systems are based on evolutionary principles (random diversification followed by selection) but their relationship to the biology in question is often less than certain (Bagley and Glass 1996).

Darwinian selection at the cellular level has been proposed as the major mechanism leading to tissue differentiation in embryogenesis (Atamas 1996; Frank 1997; Kupiec 1983, 1997; Michaelson 1993; Till 1982). Some selection models of development are strictly Darwinian in both senses that DNA sequences change and also that cells with certain genotypes die. Other "cellular Darwinism" models propose that cell–cell interactions stabilize selected patterns of gene expression from among a population of cells that have a stochastic mixture of expression patterns. Maintaining a differentiated state inside a cell requires continuous regulation

(Blau and Baltimore 1991). This requirement may be even stricter when a particular differentiated state must be passed to progeny cells.

Contemporary cellular Darwinism models are not narrowly Darwinian in three important ways. First, the phenotypic variants upon which selection acts need not be determined by DNA sequence (i.e., they can be due to variants at the level of gene expression). Second, death is not the only way out. A selected phenotype may be reinforced and a nonselected phenotype may change rather than the cell having to die. Third, the generation of variation need not be random in the sense that every variant is equally likely to come about. As discussed in the previous section, variation may be generated though a combination of stochastic and deterministic processes (colored noise in the jargon of physics). This remains true whether variants are a new DNA sequence or a new pattern of gene expression.

THE IMMUNE SYSTEM IGNORES THE CONCEPTUAL BOUNDARIES THAT SEPARATE DEVELOPMENT FROM EVOLUTION

The initial generation of somatic gene diversity of the variable region of antibodies comes about via the RAG1, RAG2-mediated combinatorial joining of pre-existing cassettes. The RAG system acts on specific DNA sequences bordering the cassettes (Cortes et al. 1996). RAG does not scramble the whole genome although it causes oncogenic translocations between sequences similar to its normal consensus (Shima-Rich et al. 1997) and also can mediate transposition of junctional sequences (Hiom et al. 1998). The mutagenesis that occurs at joining and later during somatic maturation of antibody genes is likewise focused. Other loci in the same cell, including the constant region of the antibody genes, mutate much less (Rada et al. 1997; Storb et al. 1998).

Somatically educated antibody genes encode proteins that have been matured against particular targets. Presumably, natural selection favors organisms who efficiently evolve protective somatic antibodies. The joining of unmutated germ-line cassettes already encodes antibodies that form part of the innate immune system and are the initial clones from which more fit variants are selected. Selection over many generations of the entire organism could bias the repertoire created by the combinatorial joining of germ line cassettes such that organisms are born in a high state of preparedness as well as flexibility (Casali and Schettino 1996). Different combinations of unmutated germ line cassettes isolated from two different human neonates have been found to encode antibodies that recognize the same epitope (Messmer et al. 1999) found on human sperm. Redundancy in germ-line encoding is consistent with evolutionary selection for recognition of this epitope.

As a somatic evolutionary system, the immune system generates variants in DNA sequence and selects clones that pass individual tests. From a developmental perspective, the generation of variants proceeds through an ordered series of gene expression events. The stochastic aspects of joining and mutation are limited to narrow spatial and temporal bounds (spatial meaning only a certain region of the genome and temporal meaning only during a defined period of cellular differentiation). Selection in the immune system proceeds by killing cells who fail selection or, alternatively, by inducing "failed" cells to replace their unsuccessful receptor gene (Hertz et al. 1998).

CANCER: A DISEASE OF BOTH DEVELOPMENT AND EVOLUTION

Cancer is a clonal disease. Each cancer probably results from the direct descendants of one transformed cell. Many of these descendants differ in phenotype from the original transformed cell and of course from non-transformed cells of the same organism. A major question is if and how these phenotypes are related to DNA sequence changes in differing subclones and in relation to untransformed cells of the same organism. Up until approximately the early 1960s the cause of cancer was mainly considered developmental rather than mutational (Lederberg 1958). The word "oncogene" first appears on Medline in 1969 (Huebner and Todaro 1969). The importance of oncogenes, antioncogenes, and protooncogenes to "full blown" cancer is now indisputable, but it is still not clear that changes in DNA sequence(s) tell the whole story of cancer, including its origin, progression, and metastasis.

Neoplasms are graded by their differentiation (i.e., the degree to which their cells morphologically and functionally resemble normal cells). Many neoplasms contain cells that are differentiated into several different tissue types. Teratomas in particular may contain pieces of bone, skin, and muscle, that is, representative tissues of all three embryonic layers (Cotran et al. 1994). The formation of tissues in many neoplasms implies that aberrant developmental lineages are playing a role. In the same way that many DNA sequence changes in cancers are superfluous, some developmental changes may be more causal, and others irrelevant effects of neoplastic transformation.

Some of the hereditary changes in pre-cancer and cancer cells occur via the mechanisms that operate in normal cells as they differentiate (i.e., not by changing DNA sequence, but by establishing and stabilizing patterns of gene and phenotype expression). Cell cultures derived from mouse teratomas, when transplanted into adult mice, lead to tumors. Cells from the same culture injected into mouse blastulas multiply and differentiate as normal cells to make chimeric animals.

The descendants of transformed cells participate in all tissues of the embryo and adult, including the germ line (Brinster 1974; Mintz and Illmensee 1975; Papaioannou et al. 1975; Pierce 1977). A strong interpretation of this finding is that oncogenesis is purely an alternative or aborted form of development and in principle is reversible at the level of cell differentiation (Pierce 1993).

Other cases also imply that cancer can display heredity with characteristics unexpected for DNA sequence mutations. Fox and collaborators (Fox and Radacic 1978) found that heritable resistance to purine analogs in lymphoma cell lines occurs very frequently and is easily lost during growth under nonselective conditions. Epigenetic inheritance in the form of cytosine methylation has been documented in tumors and transformed cell lines (Bird 1996; Dobrovic and Simpfendorfer 1997; Dorssers and van Agthoven 1996; Laird and Jaenisch 1996; Steinmetz et al. 1998). These particular methylation imprints are not proven involved in oncogenic transformation. However, global overmethylation has been demonstrated to play a direct role in fibroblast transformation by *fos* (Bakin and Curran 1999).

H. Pitot divides carcinogenesis into stages of initiation, promotion, and progression (Pitot 1993; Pitot and Dragan 1991). The stages of initiation and progression both involve structural changes in the genome (i.e., sequence changes small or large, whereas the stage of promotion does not). "Promotion" is considered as largely reversible and maintained with, and only with, the continuous presence of signals that bias gene expression toward cancer.

Multiple sequence mutations probably play key roles in most if not all cancers by the time they are detected clinically (Loeb 1998). p53 is mutant in more than half of cancers and many of the others are mutant in proteins that directly interact with p53 (Levine 1997). Human mismatch correction genes are oncogenes (Fishel et al. 1993). However, mice or humans harboring defective alleles of mismatch repair genes do not suffer increases for of all types of cancer (Narayanan et al. 1997).

While some DNA sequence changes are apparently necessary for cancer, not all DNA changes found in cancer are relevant. Many (but not all) transformed lineages exhibit extraordinary and global genetic instability and accumulate DNA sequence changes (including large alterations such as duplications, translocations, and aneuoploidy). New sequences are available for sequential selection (Nowell 1976) and some are important for initiation and progression, but many changes are gratuitous, resulting from clonal instability. Much of the genetic diversity seen in cancer progression is the product of neutral drift as defined by Kimura (Kimura 1991). It is a major challenge to tell the genetic changes that matter from those that came along for the ride. The best place to hide a book is in a library. Although many do, not all tumors or transformed cell lines display sequence and/or karyotype instability (Heppner and Miller 1998). Genetic stability *in vivo* is particularly important to consider because *in vitro* assays of cell-line mutagenesis depend on growth conditions (Richards et al. 1997).

Like the immune system, cancer may be a hybrid between evolutionary (stochastic and sieving) and developmental (i.e., stabilization of expression patterns) mechanisms. What would be the basis for such hybrid mechanisms in cancer? It has been reasonably hypothesized that variants on the level of gene expression may also provide substrates for somatic selection (see section on evolutionary models in development). Several lines of analysis have shown that cells of which a tumor is made display a great deal of heterogeneity for many different characteristics. Fluorescent activated cell sorting (FACS) analysis has been particularly useful for characterizing this tumor-cell heterogeneity both in the genome (Bergers et al. 1996; Devilee and Cornelisse 1994; Giaretti 1994) and of various phenotypes (Liu et al. 1998; Yeatman et al. 1989). Part of this heterogeneity is no doubt transient, and part may be stabilized via mutation. It remains to be seen if a part of the cellular heterogeneity is stabilized via physiologic and/or epigenetic mechanisms. Stabilized expression patterns in replicating cells imply selectable hereditary phenotypes even among cells that retain the same DNA sequence. This perspective and its implications for cancer initiation and progression bring certain questions into focus. First, what is the unit size for differentiated expression and for heredity of an expression pattern? Stabilized oncogenic systems may include interactions between differentiated cell types in a "tumor tissue" (Heppner and Miller 1998). Second, how stable is the expression pattern during the lifetime of the cell? Third, how stable is it through cellular generations (i.e., is it heritable)? If variability in expression among cancer cells that have the same DNA sequence is hereditary, then inherited phenotypes should be subject to selection and play a role in cancer.

THE INITIAL STATE OF ONCOGENIC TRANSFORMATION

The first heritable change in a cellular lineage that leads to a transformed clone could conceivably be either physiological or a change in DNA sequence. The edges and hybrids between these categories are of special interest. It has been argued that the first changes in cancer ought to be considered as if they are the first changes that give rise to a new species (Cairns 1981). As an alternative, the initial events might be considered as if they were the first changes that give rise to a new differentiated cell lineage. These two types of hereditary changes are conceptually distinct, but need not be independent in terms of mechanism. As reviewed above, there are physiological states predisposed for mutation and

recombination. Two cases of special relevance are the finding that DNA hypomethylation in mouse embryonic stem cells leads to enhanced mutagenesis (Chen et al. 1998) and the finding that the human mismatch correction genes themselves are preceded by iterated sequences that are likely hotspots for frameshift mutation (Malkhosyan et al. 1996).

It has been argued (Tomlinson et al. 1996) that selection for mutants rather than any elevation of mutation frequency is the dominant force in producing the cellular genotypes and phenotypes of cancer. This analysis may be correct, but it need not be the whole story. In particular, one is not sure if the turnover of cells inside a tumor is enough to support the replacement of the population by rare more-adapted types occurring at the basal mutation rate for cells in the organism. Their point is well taken that many cancers are not made up of cells that are demonstrably mutators. On the other hand, temporary states enhanced for genetic change occur through a variety of mechanisms, some of which are stabilized via DNA sequence mutation, some are physiologically stabilized, and some are fleeting. The Luria-Delbruck approach only considers mutation as a fixed probability per cell replication, but other models allow for mutation in non-growing cells be they bacteria, pre-cancerous, or transformed (MacPhee 1995; Richards et al. 1997; Rosenberg et al. 1998).

Mutations that lead to cancer initiation and progression could occur in cells that were transient mutators. By demanding that cells pass selection (e.g., growth in a particular medium or tumor formation) before they are examined one may lose the initial state that gave rise to the selected genetic or physiological inherited condition. It would be of special interest to know the mutagenic potential of a cell in real time. Currently one can learn this only in retrospect by counting mutants among its progeny. Approaches that allow for the assay of premutagenic substrates (Jackson et al. 1999; Wagner et al. 1995), such as modified or mispaired bases, and other discontinuities in DNA, may allow measurement of short-lived intermediates. A prediction is that pre-cancerous cells and cancer will be enriched for pre-mutagenic intermediates, whether or not these cells are mutators by the usual tests.

A HYPOTHESIS: MUTATOR STATES FOR EXPRESSION

As reviewed above, the probability that a cell will give rise to mutant progeny is subject to several types of modulation. Cellular Darwinism (also reviewed above) maintains that stochastic variation in phenotypic expression among cells provides differing individuals for the selection of particular phenotypes in the organismal environment. By hypothesis, and analogy to mutators at the level of DNA sequences, the rate at which a cell generates potentially selectable phenotypic variants may itself be subject to processes that alter the frequency with which alternatives are generated. A pre-cancerous condition may be one which is a "phenotypic mutator" (i.e., a cell that passes through many expression states in a short time). If the environment reinforces a state, stabilization may result. "Reinforcement" and "stabilization" are loaded terms. In principle, either could occur by physiological mechanisms at the level of cell–cell communication, or at the population level due to selection among pre-existing stable variants.

COMBINATORIES IN EVOLUTION AND DEVELOPMENT

An important mechanism for transcriptional control occurs through the sequestration of the RNA polymerase in the neighborhood of some promoters rather than others. This localization is specified via a combination of transcription factors, none of which has the binding strength itself, at physiological concentrations, to localize the polymerase (Ptashne and Gann 1997). In fact, the same level of specificity is achievable by the mechanism of allosteric modification. It has been argued that the mechanism of combinatorial localization may be favored because it is more evolvable in the sense phylogeny than allosteric modification (Ptashne and Gann 1998). This combinatoric and modular mechanism may also be optimal because it is more flexible in allowing the physiological hereditary properties needed for the differentiation of tissue-specific lineages. This is a rich context for the interplay between ontogeny and phylogeny (Herbert and Rich 1999). The alternative splicing of mRNA is also combinatorial and the same reasoning applies to it as a rich substrate for both evolution (de Souza et al. 1998) and development (Breitbart et al. 1985).

DIFFERING LINEAGE QUALITIES

Hypothesis: a single cancer may contain subclones with differing evolutionary and developmental lineage qualities. A "phenocopy" is an environmentally induced condition with the same phenotype as a "genetic" (i.e., DNA sequence-based) mutant. Some phenocopies are induced by short chemical or temperature treatments, during embyrogenesis, yet persist throughout the life of the organism (Newcomb 1990) even through many cell divisions. Transformation of a normal cell into a cancer cell is a multistep process with completion requiring between three and six mutations. Clonal outgrowth of each subpopulation is proposed to precede the next mutagenic event (Nowell 1976) and specific mutations are proposed to occur in a stereotyped sequence (Vogelstein and Kinzler 1993). Heritable change and clonal

outgrowth can occur with, or without, changing DNA sequence and, in principle, either type of inheritance could fulfill a step of cancerous transformation (Rubin 1994). Phenocopies might occur first with their eventual replacement by more stable or dramatic sequence-based modes of inheritance. It should be noted that the finding of sequence-based mutations in clinical material requires a level of sensitivity approximately 1% because the mutation is often present in only a minority of cells in a biopsy sample (Khanna et al. 1999). This is interpreted to be a consequence of normal cells infiltrating the tumor. Alternatively, biopsy samples may actually contain a mixture of cells that are transformed based on DNA sequence and other cells that are heritable phenocopies of a transformed state. Angiogenesis in development (e.g., in placentogenesis, early development), injury (stroke, heart attack) and cancer (glioblastomas have a high degree of angiogenesis) could be a part of the same "developmental program."

PLASTICITY IN MICROORGANISMS

Cancer recapitulates the controversy of plasticity in microorganisms that occurred during the rise of contemporary molecular genetics. It was a question of how much of the variability seen in microorganisms is due to malleable expression and heritable variation within a single species (Brock 1990). Some argued that there exists only one bacterial species whose phenotype differs depending on how it is cultured (Hadley 1927). These arguments did not have the benefit of our contemporary (and hopefully) clear conceptual distinctions between the modes of hereditary variation. Several concepts have often been confused: (1) random variation followed by selection (Darwinism); (2) induced mutation specific to the environment, in the extreme, Larmackism; and (3) rapid and reversible mutations within a limited repertoire (phase variation). Responsible mechanisms for particular cases include site-specific recombination, polymerase slippage at iterated sequences, and alternate patterns of methylation (Braaten et al. 1991). A fourth concept is that of stabilization and inheritance of physiological states (Novick and Weiner 1957; Ptashne 1986), or stabilization by imprinting (Grewal and Klar 1996; Klar 1998; Thaler et al. 1990) via epigenetic methylation or histone acetylation (Turner et al. 1992). The fourth numbered class of hereditary mechanisms is the major theme in multicellular differentiated organisms.

CONCLUSION

There are two broad categories of mechanism by which clonal lineages occur in biology. Evolution occurs through changes in DNA sequence and subsequent selection. Development typically does not involve changes in DNA sequence but instead proceeds via heritable patterns of gene expression. Lineage categories (evolution and development) correlate with mechanistic categories, but there are exceptions and intermixtures. The immune system of an individual mammal is the best-documented hybrid case. Based on looser evidence at present, cancer may be another.

Niels Bohr is said to have consigned true statements to two categories: ordinary truths, whose opposites are false, and deep truths, whose opposites are also deep truths (Stent 1985). The edges and crossovers among mechanisms for the stability and change of hereditary information in the lineage classes of evolution, development, immunology, and oncogenesis imply deep truths at the core of biological information processing.

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