*EPIGENETIC CONTROL SYSTEMS**

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Advances in chemical genetics in the past few years have permitted the formulation of a consistent hypothesis concerning the chemical nature of the "primary" genetic material.[1] This material is considered to consist of nucleic acid (usually deoxyribosenucleic acid) and to contain genetic information in a code of prescribed nucleotide sequences. The information is believed to be preserved during replication by a "semiconservative" reproductive mechanism, whereby each daughter strand retains a half of the parental molecule and reconstructs a complementary portion. Alterations in the genetic material are thought to come about in one of two major fashions. Mutations are the more or less random alterations in the code which result from chance substitutions in the nucleotide sequences or from gains or losses of nucleotides. Recombination also results in changes in the code, but these changes are achieved in a more orderly fashion; they require the physical association of different kinds of genetic material and are limited in their possibilities in any given instance by the nucleotide sequences in the parental materials. According to this hypothesis, the genetic material expresses its specificity through a decoding process, by which the information in the nucleotide sequences is eventually translated into, for example, amino acid sequences in proteins.

This view of the nature of the genetic material, while certainly not established in detail, finds much support in experimental studies and gains great strength from its simplicity. It permits, moreover, a clearer conceptual distinction than has previously been possible between two types of cellular control systems. On the one hand, the maintenance of a "library of specificities," both expressed and unexpressed, is accomplished by a template replicating mechanism. On the other hand, auxiliary mechanisms with different principles of operation are involved in determining which specificities are to be expressed in any particular cell. Even without specifying precisely how these other mechanisms operate, the distinction between mechanisms involving template replication and "other mechanisms" is reasonably clear, even though both are involved in determining cellular characteristics. Difficulties arise, however, when one attempts to determine whether observed differences in cellular properties are due to differences in the "primary genetic material" or to differences in other cellular constituents. Some of these difficulties can be made apparent by setting forth certain general propositions related to the supplementary regulatory systems for which evidence is now available. To simplify the discussion of these two types of systems, they will be referred to as "genetic systems" and "epigenetic systems." The term "epigenetic" is chosen to emphasize the reliance of these systems on the genetic systems and to underscore their significance in developmental processes.[2]

1. *Cells with the Same Genetic Material May Manifest Different Phenotypes.*— Although this proposition cannot be directly demonstrated to be true, abundant circumstantial evidence for its validity is available. Certainly, microbial cells grown on different substrates or cells found in different tissues of a higher organism

can be distinguished on many bases—morphological, physiological, and biochemical. Yet the available evidence makes it unlikely that these cells have changed their genetic information in the process of becoming different. The existence of phenotypic differences between cells with the same genotype merely indicates that the expressed specificities are not determined entirely by the DNA present in the cell—that other devices, epigenetic systems, regulate the expression of the genetically determined potentialities.

2. *The Genetic Potentialities of a Cell Are Expressed in Integrated Patterns.*—The determination of expression or non-expression of a particular specificity is seldom, if ever, an independent determination for each specificity stored in the "genetic library." In many cases, as in the sequential induction of enzymes, the expression of one specificity may involve the eventual expression of a series of specificities. Conversely, the expression of one specificity may make impossible the expression of other potentialities. Mutual exclusion, in a gross sense, is apparent throughout embryonic development; a cell cannot function as a nerve cell and as a liver cell at the same time. Phase variation in *Salmonella*,[3] serotype variation,[4] and mating-type variation[5] in certain ciliates provide examples of mutual exclusion in micro-organisms. Both simultaneity of expression and mutual exclusion of expression imply that intercommunication and metabolic linkage are important characteristic features of epigenetic systems.

3. *Particular Patterns of Expression Can Be Specifically Induced.*—This is, of course, a basic generalization in developmental biology; cells develop in particular ways in part at least because they occupy certain geographical locations and are exposed to certain chemical environments. Similarly, directed changes in micro-organisms, within limits set by the genotype, can be observed; induced enzyme synthesis is a special case of this phenomenon. The specific agents of induction need not be extrinsic in origin, however, but may be produced within a single cell. This proposition of specificity of induction is a corollary of the previous proposition, since integration and specific inhibition would be difficult or impossible without intracellular communication by specific agents. Moreover, a large part of the utility of epigenetic systems for micro-organisms lies precisely in their ability to respond specifically to altered environmental conditions.

4. *Epigenetic Systems Show a Wide Range of Stability Characteristics.*—Certain patterns of expression, although specifically induced, may be perpetuated in the absence of the inducing conditions. For this reason, cells with the same genotype may not only manifest different phenotypes, but these differences in expressed potentialities may persist indefinitely during cellular division in essentially the same environment. The widely held view that differentiated cells in vertebrates do not "de-differentiate" suggests that some epigenetic systems have great stability; the lack of universal agreement on this view suggests that epigenetic systems vary in stability. The observation that cells of *Escherichia coli*, adapted and unadapted to galactoside, can be maintained indefinitely in the same environment provides a recent and striking example of a stable epigenetic homeostat.[6] Other examples in unicellular forms are, again, the systems regulating serotype and mating-type specificities in the ciliates.[4, 5]

These observations create a real problem. One operational definition for "hereditary differences" has involved the indefinite perpetuation of cellular dif-

ferences during growth in the same environment. Yet instances are known in which cellular differences may be maintained in the absence of detectable genetic or environmental differences. Hence the observation of indefinite persistence of differences does not distinguish persistent homeostasis due to DNA maintenance (genetic homeostasis) from persistent homeostasis due to epigenetic regulation (epigenetic homeostasis). Moreover, great difficulty is encountered in separating, on any conceptual basis, epigenetic systems controlling differences which persist indefinitely from systems maintaining differences for shorter periods of time, and these, in turn, from systems relating to differences which disappear immediately when cells are placed together in the same environment. "Cellular memory" is not an absolute attribute. Thus the permease system mentioned above[6] shows persistence during growth under one set of environmental conditions but not under another set of conditions. Moreover, what appear to be very similar systems of control of serotype specificities in varieties 1 and 4 of *Paramecium aurelia*[4] differ considerably in their ability to maintain differences in a common environment; even within a single variety wide variations in serotype stabilities are observed, depending upon which specificities are being expressed and what cultural conditions are employed. Therefore, the separation of epigenetic regulators into two major classes, depending on their stability, would separate regulators with fundamentally similar mechanisms into two artificial categories and might even place the same system in different categories, depending on the conditions of observation. This variability in stability of a single epigenetic system may be a very common phenomenon; the major operational difficulty lies, however, in separating genetic systems from epigenetic systems under conditions of maximal stability.

5. *Some Epigenetic Devices May Be Localized in the Nucleus.*—Some attempts to characterize cellular regulatory agencies have placed considerable reliance on a geographical distinction; the genetic systems were considered to reside in the nucleus and on the chromosomes, to be stable and insulated from environmental alterations. The supplementary systems were thought to occupy the cytoplasm, to be more flexible and responsive to environmental alterations. While this distinction may have some general validity, its usefulness in particular cases may be slight. First, some of the systems of greatest interest are not amenable to the operations permitting a distinction between nuclear and cytoplasmic bases, i.e., breeding analysis or nuclear transplantation. Second, some genetic material occurs in the cytoplasm, although its common occurrence there is debatable. More serious are observations which suggest that some epigenetic control systems are located in the nucleus. The studies on nuclear differentiation in amphibian development[7] provide perhaps the most dramatic single example of this evidence. Studies on the serotypes in *Salmonella*,[3] however, go even further in suggesting that such control systems may even be localized on the "chromosomes" themselves. If such systems are so localized and particularly if they manifest considerable stability, they would behave in breeding analyses in a manner strictly comparable to genetic systems and would be indistinguishable from them on this basis alone. Certainly, when breeding analysis demonstrates a chromosomal localization of a determinant of cellular differences, strong evidence must be available to counterbalance the presumption of genetic control, but in some instances chromosomal control systems manifest so many "typical" epigenetic characteristics that their classification as such may be warranted.

This discussion then leads directly to the critical question: Can any available operation, or combination of operations, yield an unambiguous classification of cellular regulatory systems into genetic and epigenetic categories? Individually, persistence and nuclear localization have been rejected as adequate criteria. While a lack of persistence excludes a genetic basis for differences, the observation of persistence does not exclude an epigenetic basis. Although indicative, neither cytoplasmic nor nuclear localization certainly classifies a control system, since some genetic elements exist in the cytoplasm and some epigenetic systems are found in the nucleus.

If these commonly used criteria are inadequate, what other criteria can be employed? One criterion of possible utility concerns the specificity of induction of changes, but this criterion is also subject to ambiguous interpretation. Epigenetic systems are useful biological systems primarily because of their susceptibility to control; integration without specific inhibition and specific induction would be impossible. In principle, therefore, epigenetic alterations should be predictable and, under the proper circumstances, should occur with great reliability. In practice, however, a failure to induce a change with predictable results might reflect nothing more than a lack of knowledge about the proper conditions. Moreover, certain types of genetic alterations can be achieved with precision. Thus cytoplasmic genetic elements may be lost by specific environmental manipulations, and specific introduction of genetic material (as in virus infection) may not be detected as such, yet may lead to specific genetic alterations. Moreover, quantitative changes in the genetic material may be achieved by known treatments, and differential quantitative changes may occur within a single cell. One cannot even entirely exclude the possibility of specific mutagens, in the sense of agents which reliably alter particular nucleotide sequences in a particular manner, though convincing evidence for such agents is not yet available. In spite of these difficulties, one might expect epigenetic systems to be less stable and more susceptible to extrinsic control than genetic systems, and this criterion may be of some value.

Finally, the restrictions imposed on epigenetic systems by the genetic material require that the alterations which occur be limited by the information available in the genetic library. Hence one may in certain cases find suggestive evidence for epigenetic control if the number of "states" of a system is limited.

These criteria must be recognized as individually inadequate, unsatisfactory in combination, and provisional at best. Their application at the present time must involve a considerable subjective factor. Nevertheless, they constitute our chief barricade from nihilism and provide a basis for tentatively classifying several chromosomal homeostats as epigenetic.

A detailed analysis of all the systems which show presumptive epigenetic characteristics, or even those which may involve nuclear epigenetic homeostats, is not possible in this essay. Nevertheless, a few such systems and their more significant attributes will be mentioned briefly. In certain cases, where breeding analyses of somatic nuclei are not possible, a nuclear localization of control systems has been established by other means. These cases are considered to involve epigenetic control for the following reasons. The persistent nuclear alterations studied by Briggs and King,[7] in so far as they can be reckoned to be normal alterations occurring during development, were probably induced by specific, but unknown, local conditions of the embryonic environment. The nuclear alterations responsible for

mating-type differentiation in the ciliates[5] may also be specifically induced, in some cases by the cytoplasmic environment; they show, moreover, a limitation of the spectrum of changes which is directly imposed by the genotype.

The system of phase variation in *Salmonella*, studied by Lederberg and Iino,[3] has been analyzed by transductional techniques and shown to be localized on the "chromosome." The epigenetic character of the system is suggested by the relative instability of the homeostat but is indicated much more strongly by the limitation of the spectrum of changes to stable specificities controlled by two genetic loci. Some of the changes in this system have not yet been brought under direct control, but other changes appear to be directed by internal regulatory agents.

The studies of Brink and his co-workers[8] on modifications at the R locus in maize present another situation of considerable interest. Certain chromosomally localized alterations are specifically induced when particular homologous genetic elements are brought together in heterozygotes; these modifications may persist indefinitely after the inducing conditions are removed but can be reversed to a certain extent under other specific intracellular conditions. This study bears many resemblances to that of Rizet[9] on the "barrage" effect in *Podospora*, who also demonstrated a specific cellular alteration, induced in a particular heterozygote, associated with a particular genetic locus, and persistent after the heterozygous condition was resolved. Moreover, a reversal of the alteration could be achieved by exposure of the modified strain to cytoplasm of an unaltered strain. Although some of these changes in both maize and *Podospora* occurred in known heterozygotes, recombination is considered an unlikely explanation, since no detectable alterations occurred in one of the allelic loci and since the cells receiving the other locus were modified in 100 per cent of the cases. (Other alterations occurring specifically in heterozygotes are difficult to distinguish from recombination of some sort, particularly when they occur only very rarely; the possibility, however, that some rare interallelic effects—such as in "gene conversion"—are due to epigenetic alterations cannot be discounted.)

Finally, the studies of Smith and Sand[10] suggest that epigenetic systems may be responsible for some of the phenomena ascribed to "mutable genes." They extracted an unstable locus from an interspecific hybrid of *Nicotiana* which shifted frequently between two forms in both somatic and germinal tissue. A specific induction of the alterations is suggested by the fact that the alterations in the soma appear to occur at specified times in development, different for the two directions of change. The rates of change are, moreover, highly and differentially sensitive to temperature. The detection of this unstable gene in an interspecific hybrid suggests that the gene, removed from the genome of origin, is incapable of responding in a regular fashion to normal development stimuli in a foreign genome.

This discussion is based on the idea of two types of cellular regulatory systems, both capable of maintaining persistent cellular characteristics but achieving homeostasis by different means. The current concept of a primary genetic material (DNA), replicating by a template mechanism, is opposed to a homeostatic system operating by, perhaps, self-regulating metabolic patterns.[11] The details of operation in neither type of system can be precisely determined at the present time, and any attempt to discriminate between them may be premature. Certainly, an operational distinction at the present time encounters great difficulties. Never-

theless, a recognition of the existence of the two types of systems, and even the difficulties in distinguishing between them, may be useful in avoiding confusion in discussing cytoplasmic inheritance, developmental alterations, inheritance of acquired characters, mutation, and genetic recombination.

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[2] See C. H. Waddington, *Principles of Embryology* (New York: Macmillan Co., 1956).

[3] J. Lederberg and T. Iino, *Genetics*, **41**, 743, 1956.

[4] G H. Beale, *Intern. Rev. Cytol.*, **6**, 1, 1957.

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[9] G. Rizet, *Rev. cytol, et biol. végétales*, **13**, 51, 1952.

[10] H. H.Smith and S. A. Sand, *Genetics*, **42**, 560, 1957; S. A. Sand, *Genetics*, **42**, 685, 1957.

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