A Brief History of Epigenetics

Gary Felsenfeld

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health,
Bethesda, Maryland 20892–0540

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1 Introduction

The history of epigenetics is linked with the study of evolution and development. But during the past 50 years, the meaning of the term "epigenetics" has itself undergone an evolution that parallels our dramatically increased understanding of the molecular mechanisms underlying regulation of gene expression in eukaryotes. Our present working definition is "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Riggs et al. 1996). Until the 1950s, however, the word epigenetics was used in an entirely different way to categorize all of the developmental events leading from the fertilized zygote to the mature organism—that is, all of the regulated processes that, beginning with the genetic material, shape the final product (Waddington 1953). This concept had its origins in the much earlier studies in cell biology and embryology, beginning in the late 19th century, that laid the groundwork for our present understanding of the relationship between genes and development. There was a long debate among embryologists about the nature and location of the components responsible for carrying out the developmental plan of the organism. In trying to make sense of a large number of ingenious but ultimately confusing experiments involving the manipulation of cells and embryos, embryologists divided into two schools: those who thought that each cell contained preformed elements that enlarged during development, and those who thought the process involved chemical reactions among soluble components that executed a complex developmental plan. These views focused on the relative importance of the nucleus and cytoplasm in the developmental process. Following Flemming's discovery of the existence of chromosomes in 1879, experiments by many investigators, including Wilson and Boveri, provided strong evidence that the developmental program resided in the chromosomes. Thomas Hunt Morgan (1911) ultimately provided the most persuasive proof of this idea through his demonstration of the genetic linkage of several Drosophila genes to the X chromosome.

From that point onward, rapid progress was made in creating linear chromosome maps in which individual genes were assigned to specific sites on the Drosophila chromosomes (Sturtevant 1913). Of course, the questions of classic "epigenesis" remained: What molecules within the chromosomes carried the genetic information, how did they direct the developmental program, and how was the information transmitted during cell division? It was understood that both nucleic acid and proteins were present in chromosomes, but their relative contributions were not obvious; certainly, no one believed that the nucleic acid alone could carry all of the developmental information. Furthermore, earlier questions persisted about the possible contribution of the cytoplasm to developmental events. Evidence from Drosophila genetics (see below) suggested that heritable changes in phenotype could occur without corresponding changes in the "genes." This debate was dramatically altered by the identification of DNA as the primary carrier of genetic information. Ultimately, it became useful to redefine epigenetics so as to distinguish heritable changes that arise from sequence changes in DNA from those that do not.

2 Clues from Genetics and Development

Whatever the vagaries of the definition, the ideas and scientific data that underlie the present concept of epigenetics had been accumulating steadily since the early part of the 20th century. In 1930, H.J. Muller (Muller 1930) described a class of Drosophila mutations he called "ever-sporting displacements" ("ever-sporting" denoting the high rate of phenotypic change). These mutants involved chromosome translocations (displacements), but "even when all parts of the chromatin appeared to be represented in the right dosage—though abnormally arranged—the phenotypic result was not always normal." In some of these cases, Muller observed flies that had mottled eyes. He thought that this was probably due to a "genetic diversity of the different eye-forming cells," but further genetic analysis led him to connect the unusual properties with chromosomal rearrangement, and to conclude that "chromosome regions, affecting various characters at once, are somehow concerned, rather than individual genes or suppositions 'gene elements.'" Over the next 10 to 20 years, strong evidence provided by many laboratories (see Hannah 1951) confirmed that this variegation arose when rearrangements juxtaposed the white gene with heterochromatic regions.

During that period, chromosomal rearrangements of all kinds were the object of a great deal of attention. It was apparent that genes were not completely independent entities; their function could be affected by their location within the genome—as amply demonstrated by the many Drosophila mutants that led to variegation, as well as by other mutants involving translocation to euchromatic regions, in which more general (non-variegating) position effects could be observed. The role of transposable elements in plant genetics also became clear, largely through the work of McClintock (1965).
A second line of reasoning came from the study of developmental processes. It was evident that during development there was a divergence of phenotypes among differentiating cells and tissues, and it appeared that such distinguishing features, once established, could be clonally inherited by the dividing cells. Although it was understood at this point that cell-specific programming existed, and that it could be transmitted to daughter cells, how this was done was less clear.

A number of mechanisms could be imagined, and were considered. Particularly for those with a biochemical point of view, a cell was defined by the multiple interdependent biochemical reactions that maintained its identity. For example, it was suggested in 1949 by Delbruck (quoted in Jablonka and Lamb 1995) that a simple pair of biochemical pathways, each of which produced as an intermediate an inhibitor of the other pathway, could establish a system that could switch between one of two stable states. Actual examples of such systems were found somewhat later in the lac operon of Escherichia coli (Novick and Weiner 1957) and in the phage switch between lysogenic and lytic states (Ptashne 1992). Functionally equivalent models could be envisioned in eukaryotes. The extent to which nucleus and cytoplasm each contributed to the transmission of a differentiated state in the developing embryo was of course a matter of intense interest and debate; a self-stabilizing biochemical pathway would presumably have to be maintained through cell division. A second kind of epigenetic transmission was clearly demonstrated in Paramecia and other ciliates, in which the ciliary patterns may vary among individuals and are inherited clonally (Beisson and Sonneborn 1965). Altering the cortical pattern by microsurgery results in transmission of a new pattern to succeeding generations. It has been argued that related mechanisms are at work in metazoans, in which the organization of cellular components is influenced by localized cytoplasmic determinants in a way that can be transmitted during cell division (Grimes and Auferheide 1991).

### 3 DNA Is the Same in All Somatic Cells of an Organism

Although chromosome morphology indicated that all somatic cells possessed all of the chromosomes, it could not have been obvious that all somatic cells retained the full complement of DNA present in the fertilized egg. Nor until the work of Avery, MacLeod, and McCarty in 1944, and that of Hershey and Chase (1952), was it even clear that a protein-free DNA molecule could carry genetic information, a conclusion strongly reinforced by Watson and Crick’s solution of the structure of DNA in 1953. Work by Briggs and King (1952) in Rana pipiens and by Laskey and Gurdon (1970) in Xenopus had demonstrated that introduction of a nucleus from early embryonic cells into enucleated oocytes could result in development of an embryo. But as late as 1970, Laskey and Gurdon could state that “It has yet to be proved that somatic cells of an adult animal possess genes other than those necessary for their own growth and differentiation.” In the paper containing this statement, they went on to show that to a first approximation, the DNA of a somatic cell nucleus was competent to direct embryogenesis when introduced into an enucleated egg. It was now clear that the program of development, and the specialization of the repertoire of expression seen in somatic cells, must involve signals that are not the result of some deletion or mutation in the germ-line DNA sequence when it is transmitted to somatic cells.

Of course, there are ways in which the DNA of somatic cells can come to differ from that of the germ line, with consequences for the cellular phenotype: For example, transposable elements can alter the pattern of expression in somatic cells, as demonstrated by the work of Barbara McClintock and other plant geneticists. Similarly, the generation of antibody diversity involves DNA rearrangement in a somatic cell lineage. This rearrangement (or more precisely its consequences) can be considered a kind of epigenetic event, consistent with the early observations of position-effect variegation described by Muller. However, much of the work on epigenetics in recent years has focused on systems in which no DNA rearrangements have occurred, and the emphasis has therefore been on modifications to the bases, and to the proteins that are complexed with DNA within the nucleus.

### 4 The Role of DNA Methylation

X-chromosome inactivation provided an early model of this kind of epigenetic mechanism (Ohno et al. 1959; Lyon 1961); the silenced X chromosome was clearly chosen at random in somatic cells, and there was no evidence of changes in the DNA sequence itself. In part to account for this kind of inactivation, Riggs (1975) and Holliday and Pugh (1975) proposed that DNA methylation could act as an epigenetic mark. The key elements in this model were the ideas that sites of methylation were palindromic, and that distinct enzymes were responsible for methylation of unmodified DNA and DNA already methylated on one strand. It was postulated that the first methylation
event would be much more difficult than the second; once the first strand was modified, however, the complementary strand would quickly be modified at the same palindromic site. A methylation mark present on a parental strand would be copied on the daughter strand following replication, resulting in faithful transmission of the methylated state to the next generation. Shortly thereafter, Bird took advantage of the fact that the principal target of methylation in animals is the sequence CpG (Doscoic and Sorm 1962) to introduce the use of methylation-sensitive restriction enzymes as a way of detecting the methylation state. Subsequent studies (Bird 1978; Bird and Southern 1978) then showed that endogenous CpG sites were either completely unmethylated or completely methylated. The predictions of the model were thus confirmed, establishing a mechanism for epigenetic transmission of the methylation mark through semiconservative propagation of the methylation pattern.

In the years following these discoveries, a great deal of attention has been focused on endogenous patterns of DNA methylation, on the possible transmission of these patterns through the germ line, on the role of DNA methylation in silencing gene expression, on possible mechanisms for initiation or inhibition of methylation at a fully unmethylated site, and on the identification of the enzymes responsible for de novo methylation and for maintenance of methylation on already methylated sites. Although much of the DNA methylation seen in vertebrates is associated with repetitive and retroviral sequences and may serve to maintain these sequences in a permanently silent state, there can be no question that in many cases this modification provides the basis for epigenetic transmission of the state of gene activity. This is most clearly demonstrated at imprinted loci (Cattanach and Kirk 1985) such as the mouse or human Igf2/H19 locus, where one allele is marked by DNA methylation, which in turn controls expression from both genes (Bell and Felsenfeld 2000; Hark et al. 2000). At the same time, it was clear that this could not be the only mechanism for epigenetic transmission of information. For example, as noted above, position-effect variegation had been observed many years earlier in Drosophila, an organism that has extremely low levels of DNA methylation. Furthermore, in subsequent years, Drosophila geneticists had identified the Polycomb and Trithorax groups of genes, which appeared to be involved in permanently “locking in” the state of activity, either off or on, respectively, of clusters of genes during development. The fact that these states were stably transmitted during cell division suggested an underlying epigenetic mechanism.

5 The Role of Chromatin

It had been recognized for many years that the proteins bound to DNA in the eukaryotic nucleus, especially the histones, might be involved in modifying the properties of DNA. Well before most of the work on DNA methylation began, Stedman and Stedman (1950) proposed that the histones could act as general repressors of gene expression. They argued that since all somatic cells of an organism had the same number of chromosomes, they had the same genetic complement (although this was not demonstrated until some years later, as noted above). Understanding the subtlety of histone modifications was far in the future, so the Stedmans operated on the assumption that different kinds of cells in an organism must have different kinds of histones in order to generate the observed differences in phenotype. Histones can indeed reduce levels of transcript far below those commonly observed for inactive genes in prokaryotes. Subsequent work addressed the capacity of chromatin to serve as a template for transcription, and asked whether that capacity was restricted in a cell-type-specific manner. In a 1963 paper, Bonner (Bonner et al. 1963) prepared chromatin from a globulin-producing tissue of the pea plant, and showed that when E. coli RNA polymerase was added, and the resulting transcript translated in an in vitro system, globulin could be detected. The result was specific to this tissue. With the advent of hybridization methods, the transcript populations from such in vitro experiments could be examined (Paul and Gilmour 1968) and shown to be specific for the particular tissue from which the chromatin was derived. Other results suggested that this specificity reflected a restriction in access to transcription initiation sites (Cedar and Felsenfeld 1973). Nonetheless, there was a period in which it was commonly believed that the histones were suppressor proteins that passively silenced gene expression. In this view, activating a gene simply meant stripping off the histones; once that was done, it was thought, transcription would proceed pretty much as it did in prokaryotes. There was, however, some evidence that extended regions of open DNA did not exist in eukaryotic cells (Clark and Felsenfeld 1971). Furthermore, even if the naked DNA model was correct, it was not clear how the decision would be made as to which histone-covered regions should be cleared.

The resolution of this problem began as early as 1964, when Allfrey (Allfrey et al. 1964) had speculated that histone acetylation might be correlated with gene activation, and that “active” chromatin might not necessarily be stripped of histones. In the ensuing decade, there was
great interest in examining the relationship between histone modifications and gene expression. Modifications other than acetylation (methylatation and phosphorylation) were identified, but their functional significance was unclear. It became much easier to address this problem after the discovery by Kornberg and Thomas (1974) of the structure of the nucleosome, the fundamental chromatin subunit. The determination of the crystal structure of the nucleosome, first at 7 Å and then at 2.8 Å resolution, also provided important structural information, particularly evidence for the extension of the histone amino-terminal tails beyond the DNA–protein octamer core, making evident their accessibility to modification (Richmond et al. 1984; Luger et al. 1997). Beginning in 1980 and extending over some years, Grunstein and his collaborators (Wallis et al. 1980; Durrin et al. 1991), applying yeast genetic analysis, were able to show that the histone amino-terminal tails were essential for regulation of gene expression, and for the establishment of silent chromatin domains.

The ultimate connection to detailed mechanisms began with the critical demonstration by Allis (Brownell et al. 1996) that a histone acetyltransferase from Tetrahymena was homologous to yeast transcriptional regulatory protein Gcn5, providing direct evidence that histone acetylation was connected to control of gene expression. Since then, of course, there has been an explosion of discovery of histone modifications, as well as a reevaluation of the roles of those that were known previously.

This still did not answer the question of how the sites for modification were chosen in vivo. It had been shown, for example (Pazin et al. 1994), that Gal4-VP16 could activate transcription from a reconstituted chromatin template in an ATP-dependent manner. Activation was accompanied by repositioning of nucleosomes, and it was suggested that this was the critical event in making the promoter accessible. A fuller understanding of the significance of these findings required the identification of ATP-dependent nucleosome remodeling complexes such as SWI/SNF and NURF (Peterson and Herskowitz 1992; Tsukiyama and Wu 1995), and the realization that both histone modification and nucleosome remodeling were involved in preparing the chromatin template for transcription.

It was not clear how information about the state of activity could, employing these mechanisms, be transmitted through cell division; their role in epigenetic transmission of information was thus unclear. The next important step came from the realization that modified histones recruited, in a modification-specific way, proteins that could affect the local structural and functional states of chromatin. It was found, for example, that methylation of histone H3 lysine 9 resulted in the recruitment of the heterochromatin protein HP1 (Banister et al. 2001; Lachner et al. 2001; Nakayama et al. 2001). Furthermore, HP1 could recruit the enzyme (Suv39 h1) that is responsible for that methylation. This led to a model for propagation of the silenced chromatin state along the region through a progressive mechanism (Fig. 1a). Equally important, it provided a reasonable explanation of how that state could be transmitted and survive through the replication cycle (Fig. 1b). Analogous mechanisms for propagation of an active state have been proposed that involve methylation of histone H3 lysine 4 and the recruitment of Trithorax group proteins (Wysocka et al. 2005).

Different kinds of propagation mechanisms have been suggested that depend on variant histones rather than modified histones (Ahmad and Henikoff 2002; McKittrick et al. 2004). Histone H3 is incorporated into chromatin only during DNA replication. In contrast, the histone variant H3.3, which differs from H3 by four amino acids, is incorporated into nucleosomes in a replication-independent manner, and it tends to accumulate in active chromatin, where it is enriched in the “active” histone modifications (McKittrick et al. 2004). It has been proposed that the presence of H3.3 is sufficient to maintain the active state, and that after replication, although it would be diluted twofold, enough H3.3 would remain to maintain the active state. The consequent transcription would result in replacement of H3 containing nucleosomes with H3.3, thus perpetuating the active state in the next generation.

6 All Mechanisms Are Interrelated

These models finally begin to complete the connection between modified or variant histones, specific gene activation, and epigenetics, although of course there is much more to be done. Whereas these mechanisms give us some ideas about how the heterochromatic state may be maintained, they do not explain how silencing chromatin structures are first established. It has only recently become clear that this involves the production of RNA transcripts, particularly from repeated sequences, which are processed into small RNAs through the action of proteins such as Dicer, Argonaute, and RNA-dependent RNA polymerase. These RNAs are subsequently recruited to the homologous DNA sites as part of complexes that include components of the Polycomb group of proteins, thus initiating the formation of heterochromatin. There is now also evi-
Figure 1. Mechanisms for Maintaining a Pattern of DNA Methylation and a Histone Modification during DNA Replication

(a) A mechanism for maintaining a pattern of DNA methylation during DNA replication. During replication, the individual DNA strands, with a specific methylation pattern at CpG or CpxG residues, become paired with a strand of newly synthesized, unmethylated DNA. CpG on one strand has a corresponding CpG on the other. The maintenance DNA methyltransferase recognizes a hemimethylated site, and methylates the cytosine on the new strand, so that the pattern of methylation is undisturbed. (b) A general mechanism for maintaining a histone modification during replication. The modified histone tail (m) interacts with a protein binder (pb) that has a binding site specific for that modification. pb, in turn, has a specific site for the enzyme (e) which carries out that histone modification. e, in turn, can then modify an adjacent nucleosome. During replication, the newly deposited histones which are interspersed with parental histones can thus acquire the parental modification. A similar mechanism would allow propagation of histone modifications from a modified region into an unmodified one at any stage of the cell cycle.

dence that the same mechanisms are required for maintenance of at least some heterochromatic regions. In a way, these stable cyclic reaction pathways are reminiscent of Delbruck’s 50-year-old model, of a stable biochemical cycle that maintains the state of the organism.

We now know of countless examples of epigenetic mechanisms at work in the organism. In addition to imprinting at many loci, and the allele-specific and random X-chromosome inactivation described above, there are epigenetic phenomena involved in antibody expression, where the rearrangement of the immunoglobulin genes on one chromosome is selectively inhibited, and in the selection for expression of single odorant receptor genes in olfactory neurons (Chess et al. 1994; Shykind et al. 2004). In Drosophila, the Polycomb group genes are responsible for establishing a silenced chromatin domain that is maintained through all subsequent cell divisions.

Epigenetic changes are also responsible for paramutation in plants, in which one allele can cause a heritable change in expression of the homologous allele (Stam et al. 2002). This is an example of an epigenetic state that is inherited meiotically as well as mitotically, a phenomenon documented in plants but only rarely in animals (Jorgensen 1993). Much of the evidence for the mechanisms described above has come from work on the silencing of mating-type locus and centromeric sequences in Schizosaccharomyces pombe (Hall et al. 2002). In addition, the condensed chromatin structure characteristic of centromeres in organisms as diverse as flies and humans has been shown to be transmissible through centromere-associated proteins rather than DNA sequence. In all of these cases, the DNA sequence remains intact, but its capacity for expression is suppressed. This is likely in all cases to be mediated by DNA methylation, histone mod-
plementation, or both; in some cases, we already know that to be true. Finally, the epigenetic transmission of "patterns," described above for Paramecia, now extends to the prion proteins, which maintain and propagate their alternatively folded state to daughter cells.

Although this has been presented as a sequential story, it should more properly be viewed as a series of parallel and overlapping attempts to define and explain epigenetic phenomena. The definition of the term epigenetics has changed, but the questions about mechanisms of development raised by earlier generations of scientists have not. Contemporary epigenetics still addresses those central questions. Seventy years have passed since Muller described what is now called position-effect variegation. It is gratifying to trace the slow progress from observation of phenotypes, through elegant genetic studies, to the recent analysis and resolution at the molecular level. With this knowledge has come the understanding that epigenetic mechanisms may in fact be responsible for a considerable part of the phenotype of complex organisms. As is often the case, an observation that at first seemed interesting but perhaps marginal to the main issues turns out to be central, although it may take a long time to come to that realization.

References


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