1 **Introduction to epigenomics**

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A scientific man ought to have no wishes, no affections, – a mere heart of stone.
Charles R. Darwin, English naturalist (1809–82)

The present "epigenetic soft inheritance" principles are somewhat related to those proposed earlier by evolutionist Jean-Baptiste Lamarck (1744–1829), who believed that the environment plays an important role in organisms' acquisition of evolutionary characteristics. Recent neo-Lamarckian researchers now similarly believe that the environment plays a key role in a species acquiring inherited characteristics that drive variation and evolution. Many of Lamarck's theories are now being shown to be surprisingly correct. Most of the non-heritable signals reside in and are controlled by chromatin, which is the complex of DNA and protein that makes up chromosomes. Chromatin is easily visualized by cytological stains, hence its name, which literally means "colored material" and was coined by German cytogeneticist Walther Flemming in 1879 (Flemming, 1879). Cytological stains distinguish the chromatin mass into euchromatin and heterochromatin. Chromatin that presents in a tightly condensed form is also known as heterochromatin (biologically inactive), and the non-condensed or extended loop form is called euchromatin which is the transcriptionally active form.

To date several epigenetic systems in humans have been described.

X-chromosome inactivation (dosage compensation)

This is one common type of epigenetic marking which occurs during embryogenesis in female mammals. The epigenetic mark "turns off" one of the X chromosomes that the female has inherited from her parents. The purpose of inactivating one of the X chromosomes is dosage compensation which is seen in all mammals. In 1949, two Canadian scientists, Murray Barr and Ewart Bertram, identified a highly condensed structure in the interphase nuclei of somatic cells in female cats but not in male cats. Later, this structure became

known as the Barr body, which was identified as a highly condensed X chromosome (Barr and Betram, 1949). Almost a decade later, Susumu Ohno of the Beckman Institute City of Hope, Duarte, USA, proposed X-chromosome inactivation. However, in 1961, Mary Lyon (an English geneticist at the Medical Research Council's Mammalian Genetics Unit in Oxfordshire, UK) clearly described the dosage-compensation phenomenon in mammals that occurs by the inactivation of a single X chromosome in females (Lyon, 1961). At the same time Liane B. Russell of the Oak Ridge National Laboratory of the USA proposed the same theory. Today, the mechanism of X-inactivation is referred to as the *Lyon hypothesis* or *lyonization*.

Genetic imprinting

A second form of epigenetic inheritance is genomic imprinting, in which the "stamping" (expression) of the genetic information occurs according to whether it is inherited from the mother or the father. This is termed *monoallelic expression*. In other words, it is a form of gene regulation whereby some genes are silenced when inherited from the father and some are silenced when inherited from the mother. Almost a quarter of a century after the discovery of X-chromosome inactivation by Mary Lyon, three groups (Azim Surani of the University of Cambridge, UK, Bruce Cattanach of the Medical Research Council's Mammalian Genetics Unit, and Davor Solter of the Max-Planck Institute for Immunobiology, Freiburg, Germany) independently showed that the maternal and paternal genomes play different roles during early development, which they named *genomic imprinting*.

Genetic imprinting is an inheritance process that follows non-Mendelian inheritance. Forms of genomic imprinting have been demonstrated in insects, mammals, and flowering plants. At the cellular level, imprinting is an epigenetic process that can be divided into three stages: (1) establishment of the imprint during gametogenesis; (2) maintenance of the imprint during embryogenesis and in the adult somatic cells; and (3) erasure and re-establishment of the imprint in the germ cells. Genomic imprinting is an epigenetic process that involves methylation and histone modifications. These epigenetic markers are established in the germ-line and are maintained throughout all the somatic cells of an organism. Genomic imprinting involves a chemical marking process called methylation in which a methyl (-CH_3) group is added to cytosines in the DNA. Genomic imprinting is the prime example of transgenerational epigenetic inheritance because the imprint that is established in the germ-line of a parent is passed on to the offspring where it is "read" in the next generation.

Epigetic modifications and their mechanisms

DNA methylation The most widely studied epigenetic modification in humans is cytosine methylation. DNA methylation occurs almost exclusively in the context of CpG dinucleotides. The CpG dinucleotides tend to cluster in

regions called CpG islands, defined as regions of more than 200 bases with G+C content of at least 50% and a ratio of observed to statistically expected CpG frequencies of at least 0.6 (Esteller, 2008).

Histone modifications Histones are key players in the epigenetics field, and the *nucleosome* is the fundamental unit of chromatin structure, which comprises a core of eight histones (H2A, H2B, H3, and H4 grouped into two H2A–H2B dimers and one H3–H4 tetramer) around which 147 base pairs of DNA are wrapped in 1.65 spherical turns. Histone H1 is called the linker histone. It does not form part of the nucleosome but binds to the linker DNA, sealing off the nucleosome at the location where DNA enters and leaves (Kouzarides, 2007). Histones influence every aspect of DNA function. The functions of chromatin are to package DNA into a smaller volume to fit into the cell, to strengthen the DNA to allow mitosis and meiosis, and to serve as a mechanism to control gene expression. Histones that are present in nucleosomes undergo two biochemical modifications: methylation and acetylation.

Nucleosome positioning Nucleosomes are a barrier to transcription that block access of activators and transcription factors to their sites on DNA; at the same time they inhibit the elongation of the transcripts by engaging polymerases. The packaging of DNA into nucleosomes appears to affect all stages of transcription, thereby regulating gene expression (Schones *et al.*, 2008).

All of the above steps involve a battery of enzymes and protein complexes, including histone methyltransferases (HMTs), histone acetyl transferases (HATs), histone deacetylases (HDACs), histone demethylases (KDMs), histone deacetylases, DNA methyltransferases (DNMTs), DNA demethylases, and nucleosome remodeling complexes. The activity of all of these proteins could be regulated by signaling molecules. The structure and function of chromatin varies considerably as the cell progresses through the cell cycle, by participating in DNA replication, repair, and damage. In the past three Nobel Prizes have been awarded (to Thomas Morgan, 1933, Aaron Klug, 1982, and Roger Kornberg, 2006) in the field of the elucidation of the function and structure of chromatin. However, its involvement in development, differentiation, disease, and genomic imprinting is still unclear, and a mystery to biologists.

Scope of this book

This text consists of 35 chapters, grouped into six parts, and many of the aforementioned applications are described within the various sections of the book, which are summarized as follows.

Part I: Basics of chromatin biology and biochemistry

This section consists of five chapters. In the early 1940s Conrad Waddington had published important research in embryology and genetics; he realized that there

should be a synergistic relationship between these two fields (Waddington, 1939). The new field was later termed *epigenetics* (Waddington, 1942). In effect, epigenetics was extinguished in the resulting explosion of molecular genetics. The historical details and the development of epigenetics field have been well described in Chapter 2 by Robin Holliday, a pioneer molecular biologist and discoverer of DNA modification mechanisms and gene activity during development (Holliday and Pugh, 1975). Richard Katz and his colleagues in Chapter 3 describe the use of the green fluorescent protein reporter model system and siRNA screens to understand the functional networks of human epigenetic factors; this has resulted in detection of the interruption of epigenetic silencing functions in human cells. The recent upsurge of discoveries in epigenetics has revealed numerous tissue-specific DNA methylation and histone modification patterns that distinguish different parts of the human genome. It is believed that these modifications can induce global chromatin compaction or decondensation. Some functions of these modifications may be to control nucleosome stability and positioning in promoters and enhancers which critically regulate transcriptional activities. Nucleosome positioning is found among different cell types, and the interplay among transcription factors, chromatin remodelers, non-coding RNAs, and histones may play crucial roles in establishing and maintaining cell-type-specific chromatin structure. In Chapter 4, David Fisher and his colleagues summarize the results of high-throughput genome-wide mapping of nucleosomes with respect to their positioning and transcriptional regulation.

Posttranslational modifications, namely protein acetylation and methylation, are crucial in protein regulation and regulators of various epigenetic *histone code* phenomena (Allis *et al.*, 2007; Kouzarides, 2007). Proteins can be posttranslationally acetylated at their N-termini as well as at serine, threonine, and lysine residues; however, lysine acetylation is the most prevalent type of acetylation. Protein acetylation is a reversible posttranslational modification that is regulated by lysine acetyltransferases and deacetylases. Chemical reporter and mass-spectrometry-based proteomics strategies are the two major technological developments in recent years towards the analysis of posttranslational modifications. Therefore, to understand the protein acetylation and methylation mechanisms in the context of epigenetic regulation, Howard Hang and his colleagues used chemical reporter and mass-spectrometry methods as described in Chapter 5 of this book.

In Chapter 6, Takashi Nagano summarizes recent findings on the role of long non-coding RNAs in epigenetic silencing. Our knowledge of RNA has continuously expanded since then through identification and analyses of various distinct RNAs (including ribosomal RNA, transfer RNA, small nuclear RNAs, small nucleolar RNAs, and microRNAs). Each of these RNA classes has distinct features of its own, and the members of each class are thought to share similar function. In the 1990s several long non-coding RNAs (lncRNAs) were identified including: *Xist* and *Airn* and *Kcnq1ot1* that play essential roles in regulating transcriptional silencing of other genes. *Xist* is essential for X-chromosome inactivation in mammalian female cells (Plath *et al.*, 2003).

Part II: Epigenomic imprinting and stem cells

This section consists of seven chapters detailing the molecular processes that are involved in the genomic imprinting, the fundamental phenomenon occurring in embryos. In the early hours of life, the parental genomes of highly specialized germ cells get reprogrammed in order to comply with the totipotency state of the resulting zygote, which later on upon further development gives rise to all the cells of a multicellular organism. Two of the striking events happening in the developing zygote are the processes of DNA methylation and demethylation. Although the biological significance and mechanism of DNA methylation has been explored by experiments in transgenic mice, the mechanism of DNA demethylation is still not understood. DNA demethylation is not the only player in epigenetic reprogramming – histone modifications, histone variants, and small RNAs also contribute in leading mammals through proper development. In Chapter 7, Jorn Walter and his colleagues emphasize the importance of DNA demethylation in the zygote in order to better understand epigenetic reprogramming. Histone modifications largely take place on histone N-termini, regulating access to the underlying DNA. Histone proteins and their associated covalent modifications can alter chromatin structure, and determine how and when the DNA packaged in the nucleosomes is accessed, leading to the histone code hypothesis. In Chapter 8 Kim *et al.* describe the importance of histone modifications of lineage-specific genes in embryonic stem cells during differentiation.

The status of the X chromosome reflects major epigenetic instability of human embryonic stem cells (hESCs). While most studies on the epigenetic stability of hESCs have focused on imprinted loci and X-inactivation status, some have addressed the epigenetic variation at gene promoters in the rest of the genome, especially CpG-rich promoters. Normally, human pluripotent stem cells do not stand in a defined epigenetic state. Multiple variations can occur in the epigenome of hESCs along passages and within different cell lines specially reflecting a tremendous epigenetic plasticity of hESCs. Female pluripotent stem cells can harbor various X-chromosome patterns which are not stably maintained throughout numerous cell divisions. Human pluripotent stem cells (hPSCs) potentially stand as promising therapeutic tools for degenerative diseases. Through their capacity to differentiate to any cell type, they offer the possibility of a renewable source of replacement cells to treat various diseases including Parkinson's and Alzheimer's diseases. In Chapter 9, Vallot and Rougeulle elegantly describe the epigenetic stability of hPSCs.

Stem cells are undifferentiated cells with self-renewal and differentiation potential. Among stem cells, embryonic are considered as the best for cardiac regeneration. In the last decade another group of somatic stem cells, derived from adipose tissue, has been studied. In Chapter 10, Pasini *et al.* describe the features of adipose-derived stem cells, how to isolate them from lipoaspirates, and how they differentiate into cardiomyocytes. This chapter focuses especially on the epigenetic modifications (impact of CpG methylation) that influence the cells' commitment towards a cardiac phenotype. Complex gene regulatory networks control the acquisition and maintenance of cellular phenotype and function. In order to

identify the molecular hallmarks of "stemness" it is a prerequisite to understand their transcriptional regulatory pathways or "epigenomic signatures." Epigenetic modifiers are recruited by transcription factors, and the transcription factor position is determined by the epigenetic status of the target gene chromatin. Therefore, it is important to identify factors that act to coordinate regulation of the transcriptome and epigenome. In Chapter 11, Bithell and Buckley discuss the involvement of transcription factors in the regulation of the epigenome, and provide an example of repressor element 1 silencing transcription factor (REST), which is a key neural regulator that balances stem-cell maintenance and differentiation.

As we mentioned earlier, many epigenetic elements such as DNA methylation, histone modifications, and microRNAs play crucial roles in gene regulation. MicroRNAs (miRNAs) are short sequences of RNA, 21–4 nucleotides in length, which play an integral role in the regulation of protein expression. Several such tiny microRNAs have been identified in stem cells. These tiny molecules play crucial roles in the gene network(s) involved in embryonic stem-cell maintenance, proliferation, differentiation, self-renewal, and pluripotency. In Chapter 12, Orlanski and Bergman summarize the importance of these tiny molecules in stem-cell biology and how these small molecules will likely have great impact on the use of stem-cell therapeutics. DNA replication is a process fundamental to cell proliferation, and occurs only once per cell cycle in eukaryotic cells. Additionally, after each DNA replication, the epigenetic information (such as DNA methylation and histone modifications) that constitutes the memory and the identity of the cells also is replicated. Well-regulated DNA replication is essential for normal development of an organism because the entire genome must faithfully be duplicated during a single cell cycle. Failure to do so may result in over-replication or under-replication of the genome giving rise to various abnormalities. Likely, these functional constraints might have caused eukaryotes to evolve complex tissue-specific replication programs. Thus, the timing during S phase at which each DNA segment replicates is critical for the transmission of *epigenetic memory*. The timing of replication affects gene expression or the probability of gene activation. In Chapter 13, Mukhopadhyay and Bouhassira emphasize the importance of the timing of replication in the context of epigenetic memory using genome-wide *epigenetic profiling* studies.

Part III: Epigenomic assays and sequencing technology

This section consists of seven chapters that emphasize the technological platform used to study epigenetic phenomena. A number of technologies (such as methylation-specific restriction enzyme digestion, methylation-dependent fragment separation, bisulfite DNA sequencing, pyrosequencing, and MALDI mass spectrometry) have evolved in recent years that have enhanced our abilities to identify and characterize epigenetic modifications on a genome-wide scale, and these developments have facilitated comparative studies to elucidate the functional relationship between epigenetic modifications and gene expression. The most widely studied epigenetic modification in humans is cytosine methylation. DNA methylation occurs almost exclusively in the context of CpG dinucleotides.

The conversion from the unmethylated to the methylated state has been coined an *epigenetic transition*. There are about 29 million CpG dinucleotides present in the mammalian genome. Broadly speaking there are two categories of CpG dinucleotides – clustered and unclustered. Clustered CpG dinucleotides are found primarily within and near gene loci in the mammalian genome – termed *CpG islands*. These CpG islands mediate a variety of biological processes such as gene expression, X-chromosome inactivation, imprinting, cellular differentiation, aging, and chromatin structure.

Technically, it is a quite challenge to discriminate accurately between the unmethylated and methylated states. One of such techniques, the protein-based affinity-capture method, is discussed by Acevedo *et al.* in Chapter 14 of this book. The greatest potential of this technique lies in its application in the clinical setting where it can be used to detect the methylation status of clinically significant targets that will help in the development of diagnostic or therapeutic assays. During the past decade the development of chromatin immunoprecipitation (ChIP) technologies and more recently the next-generation sequencing (ChIP–seq) technologies have emerged as the predominant methodologies for high-throughput epigenome-wide mapping. In order to provide complete genome coverage using ChIP–chip studies, typically 10^8 cells of starting material are required (which is difficult to get from stem cells or cancer tissues), and large sets of microarrays. To overcome this obstacle, a novel ChIP–chip technology, ChIP–DSL (ChIP–DNA selection and ligation) has been developed, which is discussed in Chapter 15 by Falk. ChIP–DSL technology provides increased sensitivity and utilizes a single microarray, made it adaptable to achieve rapid global profiling of epigenetic modifications.

Aberrant CpG methylation correlates in mammals with many diseases like cancer and developmental disorders. Therefore, there is a need to develop potential diagnostic marker assays to quantify DNA methylation especially at the individual CpG positions. Sanger sequencing has been a valuable technology to elucidate DNA methylation patterns; however, it is cumbersome and expensive. On the other hand, pyrosequencing, a real-time sequencing method (Ronaghi *et al.*, 1998), is cost-effective and sensitive for quantifying minor differences in modified CpG dinucleotides. Chapter 16, written by Löffert *et al.*, provides an overview of the development of high-resolution CpG methylation assays using such a pyrosequencing platform. Scientists at Illumina have developed a low-cost, high-throughput, and genome-wide DNA methylation profiling technology using the Illumina BeadChip platform. Nevertheless, in Chapter 17, Bibikova *et al.* have shown that universal arrays can access individual CpG sites across both CpG islands and genomic regions with low CpG density, and therefore give a good overview of epigenetic profiles on a genome-wide scale. Capillary electrophoresis (CE) can also be used for DNA methylation analysis. Scientists from Applied Biosystems have developed CE-based DNA methylation analysis for global analysis. Using labeled primers in the polymerase chain reaction (PCR) followed by separation on capillaries Schroeder *et al.* have described how to differentiate methylated and unmethylated gDNA, as discussed in Chapter 18.

In Chapter 19, Mingzhi Ye's team from the Beijing Genomics Institute evaluate five high-throughput sequencing methods (including BS–seq, MeDIP–seq, MBD–seq, MeDIP–BS, and ChIP–BS) in the analysis of DNA methylation at the chromatin level, and conclude that ChIP–BS method works efficiently. Very recent methods such as single molecular DNA sequencing technology will guide us into a new direction to unravel the secrets of epigenome/methylomes. In Chapter 20 Tajbakhsh and Gertych introduce a novel cytometric approach termed "Three-dimensional quantitative DNA methylation imaging." This method extracts fluorescence signals from three-dimensional images of chromatin texture in the nuclei of thousands of cells in parallel.

Part IV: Epigenomics in disease biology

This section consists of six chapters. It is increasingly being recognized that epigenetic abnormalities are critical to disease pathogenesis. Studies of epigenetic changes associated with different conditions can not only improve our understanding of the biology of the diseases and hold great promise for improving their management, but also be invaluable for providing insights into basic aspects of epigenetic regulation. The first example of a human disease with an epigenetic mechanism was cancer. In 1983, widespread loss of DNA methylation was observed in colorectal cancers compared with matched normal mucosa from the same patients (Feinberg and Vogelstein, 1983). Gene silencing is a major epigenetic gene-inactivation mechanism, by DNA methylation of the promoter region, and is involved in the initiation and progression of cancer. At present, cancer is by far the best-studied disorder with respect to epigenetic abnormalities. Several books on epigenetics by others have detailed its prominence in cancer biology. This book provides recent glimpses on the epigenome-wide studies on a few other cancers along with colorectal cancer.

For classification of cancer cases using DNA methylation data, a subset of colorectal cancer was found to show accumulation of CpG island methylation, the so-called "CpG island methylator phenotype"; this is described in detail by Hazra and Ogino in Chapter 34. Genome-wide approaches to searching for aberrantly methylated regions in cancer have been developed since 1993. in Chapter 21 Kaneda summarized cancer classification and genome-wide approaches in order to identify novel tumor-suppressor/inactivated genes and methylation markers. Colorectal cancer is the third leading cause of cancer death in both the USA and Europe. This cancer is thought to arise from pluripotent stem cells located in intestinal crypts which can develop into aberrant crypt foci and premalignant adenomas of which about 5–6% will develop into a carcinoma with invasive and metastatic potential. Nowadays colorectal cancer is one of the best-studied malignancies and provides an excellent model for the study of the complexity of epigenetics and its driving role in colorectal carcinogenesis. In Chapter 22 Derks and van Engeland outline the current knowledge of promoter CpG-island hypermethylation in colorectal cancer and the promising role of this biomarker for early disease detection, and the prediction of prognosis and response to therapy.

Urothelial carcinoma of the bladder is the third most prevalent cancer and one of the most expensive to treat. The etiology of the disease is acquired carcinogen exposure with little or no familial component. In general, low-grade tumors are characterized by infrequent aberrant hypermethylation, DNA hypomethylation, and the down-regulation of microRNAs, whereas high-grade tumors have extensive promoter hypermethylation and upregulation of many microRNAs. Numerous epigenetic alterations have been observed in this cancer, and these represent potential biomarkers or therapeutic targets due to their reversible nature. In Chapter 23, Dudziec and Catto provided an overview of this cancer. A number of animal studies linking environmental exposure to changes in epigenetic modifications such as DNA methylation suggest that changes in phenotype can arise from the environment via changes in the epigenome. The link between environment and epigenetics is becoming clearer in cancer; one important area is childhood cancer. Genome-scale analysis offers an unbiased approach in cataloging DNA methylation changes associated with disease and could be readily applied to childhood cancer. The clear differences between adult and childhood cancers preclude direct extrapolation of findings in adult studies to their childhood counterparts. Therefore there is a need to investigate the epigenomes of childhood cancer in addition to current efforts. In Chapter 24, Wong and Ashley emphasize the importance of genome-wide studies in childhood cancer.

Epigenetic chromatin regulation is crucial for myogenesis and muscle regeneration. Muscular dystrophies are a group of hereditary muscle diseases marked by muscle weakness and loss of muscle tissue. Facioscapulohumeral muscular dystrophy is one of the most common muscular dystrophies; identification of disease-specific genes for this disease is a quite challenge. Development of therapies that control the epigenetic chromatin alterations, both to stimulate muscle regeneration and to alleviate the pathological changes, is an important direction of research. In addition, therapeutic epigenetic manipulation may be of clinical value in the treatment of muscular dystrophies. Yokomori and his colleagues in Chapter 25 discuss their discovery of epigenetic changes in this condition, in the muscle and name it as an "epigenetic abnormality" disease.

The protein lysine acetylation is one of the important covalent modifications, which was first observed on histones and later on non-histone proteins. Although histone acetylation was originally reported by Vincent Allfrey in 1964 (Allfrey *et al.*, 1964), the first histone acetyltransferase (GCN5) was identified from *Tetrahymena* by David Allis's group in the mid-1990s (Brownell and Allis, 1995). This was followed by the identification of several acetyltransferases, most of which are positive regulators of transcription. Nucleosomal histone acetylation has been considered to be an indispensable component of transcriptionally active chromatin. This "acetylation" is an integral component of a code or the "epigenetic language" rather than a mere "mark." The small molecule modulators of acetyltransferases could be useful both as biological probes to elucidate the epigenetic language of cellular functions as well as therapeutic tools to target disease conditions. In Chapter 26 Selvi *et al.* summarize the roles of histone

acetyltransferases and all the possible methods available to develop histone acetylation drugs and therapeutics.

Epigenetic changes often precede disease pathology, making them valuable diagnostic indicators for disease risk or prognostic indicators for disease progression. Several inhibitors of histone deacetylation or DNA methylation are approved for hematological cancers by the US Food and Drug Administration (FDA) and have been in clinical use for several years. More recently, histone methylation and microRNA expression have gained attention as potential therapeutic targets. A key challenge for future epigenetic therapies will be to develop inhibitors with specificity to particular regions of chromosomes, thereby reducing side effects. The identification of histone demethylase enzymes has opened a new frontier in the study of dynamic epigenetic regulation. Recently, it has become clear that histone methylation contributes to maintaining the undifferentiated state of embryonic stem cells and to the epigenetic landscape during early development. The involvement of histone demethylase enzymes in disease also provides a unique opportunity for pharmacological intervention by designing small-molecule inhibitors that exploit the structure and enzymatic reaction mechanisms of these newly discovered enzymes to counteract their function. Indeed, some small-molecule inhibitors have already been identified. Hopefully, these will help to explore how dynamic histone methylation contributes to normal biological functions and disease.

Part V: Epigenomics in neurodegenerative diseases

This section consists of five chapters. The central nervous system is one of the most complex systems in humans. Recent studies have shed some light on the relationship between epigenetic alterations and neurodegenerative and/or neurological diseases such as: Rett syndrome, Rubinstein–Taybi syndrome, ATRX syndrome, fragile X syndrome, amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis, Parkinson's and Huntington's diseases, and congenital myotonic dystrophy. Epigenetic alterations are likely to be found in other disorders, e.g., autoimmune, cardiovascular, and metabolic diseases. As noted by Andrew Feinberg of the Johns Hopkins University, "*epigenomics provides the context for understanding the function of genome sequence, analogous to the functional anatomy of the human body provided by Vesalius a half-millennium ago*" (Feinberg, 2010).

Neurodevelopmental diseases (such as autism, schizophrenia, depression, and Alzheimer's disease) are complex disorders that likely arise from the interaction of alleles at multiple loci with environmental factors. However, additional information that affects phenotype is encoded in the distribution of epigenetic markers, including DNA methylation and histone modifications. It is also the fact that epigenetic phenomena have been reported in dynamic regulation of DNA methylation within differentiated neurons of the human cerebral cortex throughout development, maturation, and aging. Epigenetic alterations at selected genomic loci may affect social cognition, learning and memory, and stress-related behaviors and contribute to aberrant gene expression in a range of neurodevelopmental disorders. In Chapter 27, Haghighi *et al.* outline stable histone methylation