PREFACE

These two volumes of *Methods of Enzymology* cover the rapidly developing field of Epigenetics. The central dogma of molecular biology, first proposed by Francis Crick in 1956, provided a framework for understanding the transfer of genetic information, which flows from DNA to RNA to protein. However, in the early 1980s, it was discovered that methylation of the DNA can change its function, hinting that genetic information transfer could be altered in other ways. In the 1990s, it was discovered that gene function could indeed be altered in many other ways and the modern field of Epigenetics was born. Epigenetics, a term first coined by Conrad Waddington in 1942 as "the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being," is now known as the study of heritable changes in gene expression due to internal or environmental signals that results in the change of cellular function or physiological phenotype, but that is not caused by changes in the genetic information. An example of epigenetic regulation is cell differentiation, whereby cells of an organism with identical genetic information, such as cells of the nose, eyes, and hair, carry out different cellular functions.

At the heart of epigenetics is the regulation of chromatin, the packaged form of DNA. The building blocks of chromatin are nucleosome core particles containing about 146 bp of DNA wrapped around an octamer of histone proteins, two copies each of histones H2A, H2B, H3, and H4. Chromatin mediates all DNA-templated events, including DNA transcription, replication, and repair, and is regulated by many proteins and noncoding RNAs. The proteins that regulate chromatin include posttranslational modification (PTM) "writer" enzymes, "eraser" enzymes that remove these PTMs, and "reader" proteins that bind chemically modified histones or DNA. PTMs of DNA include methylation at the 5 position of cytosine and various oxidation states of 5-methyl-cytosine. PTMs of histones include acetylation, methylation, and ubiquitination on lysine residues; methylation and citrullination of arginine residues; and phosphorylation of threonine and serine residues. ATP-dependent chromatin-remodeling enzymes that function to reposition nucleosomes within chromatin; histone chaperone proteins that insert or eviction of histone variants in and out of chromatin, respectively; and noncoding RNA molecules also contribute to chromatin regulation.

Chromatin-regulatory proteins work together to mediate epigenetic regulation, with ramifications for cellular function and physiological phenotype, and over the last decade, it has become apparent that the dysfunction of epigenetic regulators can drive many diseases including metabolic and neurodegenerative disorders and various cancers. Over the last decade, we have seen significant progress on understanding the molecular mechanisms underlying epigenetic regulation leading to new insights into cellular function and physiological phenotype, the development of new technologies, and the development of small-molecule probes to study these biological processes and small epigenetic drugs that are currently in clinical trials to treat disease.

The remarkable progress in the field of epigenetic research that has occurred over the last two decades is highlighted in these two volumes of *Methods of Enzymology*, entitled Enzymes of Epigenetics. In Volume 1, Chapters 1 through 5 cover *Chromatin Structure and Histones*. This includes strategies for in vitro chromatin assembly (Chapter 1) and the assembly of protein–chromatin complexes (Chapter 2) for biochemical, biophysical, and structural studies, preparation of recombinant centromeric nucleosomes with and without nonhistone centromere proteins (Chapter 3), functional characterization of histone deposition by histone chaperones (Chapter 4), and methods to study nucleosome sliding by ATP-dependent chromatin-remodeling enzymes (Chapter 5).

In Volume 1, Chapters 6 through 13 cover *Posttranslational Histone Modification Enzymes and Complexes*. This includes in vitro activity assays for MYST histone acetyltransferases and adaptation for high-throughput inhibitor screening (Chapter 6), and the preparation, biochemical analysis, and structure determination of the classical (Chapter 7) and sirtuin (Chapter 8) histone lysine deacetylases, SET domain histone methyltransferases (Chapter 9), LSD1/KDM1A (Chapter 10), and JmjC (Chapter 12). Also included are chapters on LSD1 histone demethylase assays and inhibition (Chapter 11) and preparation and analysis of native chromatin-modifying complexes (Chapter 13).

In Volume 1, Chapters 14 and 15 cover *Histone Modification Binders* and include the preparation, biochemical analysis, and structure determination of the acetyl-lysine reader bromodomains (Chapter 14) and the readers of the methyllysine mark (Chapter 15).

In Volume 1, Chapters 16 through 20 cover *DNA Modifications and Nucleic Acid Regulators*. This includes quantification of oxidized 5-methylcytosine bases and TET enzyme activity (Chapter 16), characterization of how

DNA modifications affect DNA binding by C_2H_2 zinc finger proteins (Chapter 17), regulation of chromosome ends by the riboprotein telomerase complex (Chapter 18), detection and analysis of long noncoding RNAs (Chapter 19), and identification of centromeric RNAs involved in histone dynamics in vivo (Chapter 20).

In Volume 2, Chapters 1 through 8 cover *Epigenetic Technologies*. This includes identification and quantification of histone PTMs using high-resolution mass spectrometry (Chapter 1), substrate specificity profiling of histone-modifying enzymes using peptide microarray (Chapter 2), an open-source platform to analyze such microarrays (Chapter 3), chemical biology approaches for characterization of epigenetic regulators (Chapter 4), mapping lysine acetyltransferase–ligand interactions by activity-based capture (Chapter 5), methods for investigating histone acetylation stoichiometry and turnover rate (Chapter 6), semisynthesis of acetylated and sumoylated histone analogs (Chapter 7), and an IF-FISH approach for covisualization of gene loci and nuclear architecture in fission yeast (Chapter 8).

In Volume 2, Chapters 9 through 11 cover *Small-Molecule Epigenetic Regulators* and include the preparation and analysis of sirtuin deacetylase inhibitors (Chapter 9) and activators (Chapter 10) and methyltransferase inhibitors (Chapter 11).

In Volume 2, Chapters 12 through 15 cover *Epigenetics and Biological Connections* and include exploring the dynamic relationship between cellular metabolism and chromatin structure using SILAC-mass spec and ChIP-sequencing (Chapter 12), proteomic methods to investigate the dynamics of histone turnover in the central nervous system (Chapter 13), ChIP-seq techniques to map the epigenome of senescent cells (Chapter 14), and exploiting chromatin biology to understand immunology (Chapter 15).

I expect that these volumes will be a useful resource for investigators in the epigenetics field as well as those outside of the epigenetics field who would like to incorporate epigenetics into their own research programs.

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