

Contents

List of Contributors	xvii
Foreword	xxiii

CHAPTER 1 The State of the Art of Epigenetic Technologies 1 *Y. George Zheng*

1.1 Epigenetics and Chromatin Function	1
1.2 Mechanisms of Epigenetic Regulation.....	2
1.3 Technologies are Critical for the Advancement of Epigenetic Discovery	8
1.4 Epigenetic Inhibitors as Novel Therapeutics	11
1.5 Perspective	12
Acknowledgments	14
References	14

CHAPTER 2 Technologies for the Measurement and Mapping of Genomic 5-Methylcytosine and 5-Hydroxymethylcytosine 19 *Jolyon Terragni and Sriharsa Pradhan*

2.1 Introduction.....	19
2.2 Measuring Genomic Levels of 5-Methylcytosine and 5-Hydroxymethylcytosine	20
2.3 Locus-Specific Analysis of 5-Methylcytosine and 5-Hydroxymethylcytosine	23
2.4 Technologies for Genome-Wide Analysis of 5-Methylcytosine and 5-Hydroxymethylcytosine	26
2.4.1 DNA Microarray Approaches	26
2.4.2 Next-Generation Sequencing Approaches	28
2.5 Conclusions.....	34
Acknowledgments	35
References	35

CHAPTER 3 High-Throughput Sequencing-Based Mapping of Cytosine Modifications..... 39 *Alison I. Bernstein and Peng Jin*

3.1 Introduction.....	39
3.2 Cytosine Modifications.....	40
3.2.1 5-Methylcytosine	40

3.2.2	5-Hydroxymethylcytosine	40
3.2.3	5-Formylcytosine and 5-Carboxylcytosine	41
3.3	High-Throughput Sequencing Methods for Detecting Cytosine Modifications.....	42
3.3.1	Genome-Wide Mapping of Cytosine Modifications.....	42
3.3.2	Single-Base Resolution Methods of Detecting Cytosine Modifications.....	45
3.4	Conclusions.....	49
	References	49
CHAPTER 4	Application of Mass Spectrometry in Translational Epigenetics	55
	<i>Xiaoshi Wang, Simone Sidoli, and Benjamin A. Garcia</i>	
4.1	Introduction.....	55
4.2	Applications of Mass Spectrometry in Epigenetic Research	58
4.2.1	Principles of Mass Spectrometry in Proteomics	58
4.2.2	Major Approaches for Mass Spectrometry-Based Histone PTM Analysis	60
4.2.3	Bioinformatics for Histone Code Analysis	67
4.2.4	Quantification and Dynamics of Histone PTMs by Mass Spectrometry	68
4.3	Conclusion	72
	References	73
CHAPTER 5	Techniques Analyzing Chromatin Modifications at Specific Single Loci.....	79
	<i>Xiangyun Amy Chen, Jinqun Sun, and Yanming Wang</i>	
5.1	Gene Expression Pattern Is Related to the Covalent Modification of Core Histones	81
5.2	Heterochromatin Is Tightly Packed to Repress Gene Expression via Interaction of HP1 and Histone H3 Lys9 Methylation	81
5.2.1	Position Effect Variegation: A Classical Epigenetic Phenomenon to Understand Chromosome Organization and Gene Expression.....	81
5.2.2	Heterochromatin Formation Depends on Histone H3 Lysine 9 Methylation and HP1.....	83
5.2.3	PEV Screening: Using White Gene as a Reporter and the P Element Insertion as a Mutagen	83
5.3	In Situ Observation of Chromatin Reorganization During Transcription Activation.....	83
5.3.1	Chromatin Is Reorganized During Transcription Activation	83
5.3.2	Lac Repressor System as a Powerful Tool for Live Cell Imaging to Investigate Factors that Regulate Cell Cycle Progression	84

5.3.3 Polytene Chromosomes in <i>Drosophila</i> Provide Convenient Way to Observe Chromatin Remodeling	86
5.4 Chromatin Immunoprecipitation	87
5.4.1 ChIP Method Development and Application.....	87
5.4.2 N-ChIP Is Popular Method to Observe Histone Modifications at Specific Loci.....	87
5.4.3 Comparison of ChIP-PCR, ChIP-Chip, and ChIP-Seq.....	89
5.4.4 ChIP Troubleshooting.....	90
5.4.5 Limitations of the ChIP Method	90
5.5 Cross-Linking Chromosome Immunoprecipitation as a Powerful Tool to Investigate Sequential Recruitment of Histone Acetyltransferase and SWI/SNF Chromatin-Remodeling Complex to the Promoters	91
5.5.1 Histone Acetyltransferases and Chromatin-remodeling Complex (Such as SWI/SNF) Required for Gene Activation.....	91
5.5.2 X-ChIP Reveals Sequential Recruitment of HATs and SWI/SNF-Remodeling Complex at Different Promoters	91
5.6 Convergence of Bioinformatics and Biophysics Techniques at Observation in Live Single Cell.....	93
5.6.1 Single-Cell Live Imaging Reveals Function of Nucleosome-Depleted Region in Gene Activation	93
5.7 Future Perspectives: Epigenome Engineering and Manipulating at Specific Genomic Loci in Mammalian Cells	95
5.7.1 PAD4 Is a Histone Arg Deiminase	95
5.7.2 CRISPRs Are Promising Tools to Edit the Epigenome at Specific Genomic Loci.....	95
References	97

CHAPTER 6 Comprehensive Analysis of Mammalian Linker-Histone Variants and Their Mutants 101

Chenyi Pan, Yunzhe Zhang, and Yuhong Fan

6.1 Introduction.....	101
6.2 Linker Histone H1 and Its Variants	102
6.3 Expression Analysis of Mammalian Linker Histone H1 Variants	104
6.3.1 Analysis of H1 Gene Transcripts by Quantitative Reverse Transcription PCR	105
6.3.2 HPLC Analysis of Linker Histones	107
6.4 Genetic Analysis of H1 Variants by Gene Inactivation	108
6.4.1 Derivation of Triple and Single H1 Knockout Embryonic Stem Cells.....	109
6.4.2 Differentiation of H1 Knockout Embryonic Stem Cells	109
6.5 In Vivo Tagging and Genome-Wide Mapping of H1 Variants	110

6.5.1 Embryonic Lethality in H1c/H1d/H1e Triple Null Mice Is Rescued by FLAG-H1d	112
6.5.2 Mapping H1 Variants in ESCs	113
6.6 Mutation Analysis of Histone H1	116
6.7 Conclusion	119
References	119

CHAPTER 7 Crystallography-Based Mechanistic Insights into Epigenetic Regulation

Shuai Zhao and Haitao Li

7.1 Introduction.....	125
7.2 Development of X-Ray Crystallography.....	127
7.3 Key Epigenetic Projects Solved by X-Ray Crystallography.....	129
7.3.1 Elucidation of the Fundamental Building Blocks of Chromatin.....	129
7.3.2 Creation of Epigenetic Modification Pattern by Epigenetic Modifiers	130
7.3.3 Decoding the Epigenetic Code with Reader Modules.....	135
7.3.4 Governing Chromatin/Nucleosome Dynamics by Epigenetic Chaperones and Remodelers	137
7.4 Application of X-Ray Crystallography in Epigenetic Drug Discovery	138
7.4.1 Targeting Epigenetic Modifiers	138
7.4.2 Targeting Epigenetic Readers	140
7.5 Fragment-Based Drug Discovery	141
7.6 Conclusion	143
Acknowledgments	143
References	143

CHAPTER 8 Chemical and Genetic Approaches to Study Histone Modifications

Abhinav Dhall and Champak Chatterjee

8.1 Eukaryotic Chromatin and Histones	149
8.2 The Challenging Diversity of Histone Posttranslational Modifications.....	150
8.3 A Chemical Biology Approach to Investigate Histone Modifications	152
8.4 Strategies of Native Chemical and Expressed Protein Ligation	152
8.5 Thialysine Analogs of Methylated and Acetylated Histones	155
8.6 Genetic Incorporation of Modified Amino Acids in Histones	157
8.7 Biochemical and Biophysical Studies of Histone Ubiquitylation	158
8.8 Biochemical and Biophysical Studies of Histone Methylation.....	160
8.9 Outlook	162
Acknowledgments	163
References	163

CHAPTER 9 Peptide Microarrays for Profiling of Epigenetic Targets 169*Antonia Masch, Ulf Reimer, Johannes Zerweck, and Mike Schutkowski*

9.1	Introduction.....	169
9.2	Applications of Histone Peptide Microarrays.....	171
9.2.1	Profiling of Binding Specificity of Histone Code Readers.....	171
9.2.2	Profiling of Substrate Specificity of Histone Code Writers.....	177
9.2.3	Profiling of Substrate Specificity of Histone Code Erasers.....	180
9.3	Conclusion and Outlook.....	182
	Acknowledgments.....	182
	References.....	182

CHAPTER 10 Current Methods for Methylome Profiling 187*Minkui Luo*

10.1	Introduction.....	188
10.2	Labeling Protein Methylation.....	190
10.2.1	Challenges for Methylome Profiling with Conventional Methods.....	191
10.2.2	Methylome Labeling with Isotopes Inside Living Cells.....	192
10.2.3	Chemical Labeling of Proteome-Wide PMT Substrates <i>in Vitro</i> and within Living Cells.....	194
10.3	Recognizing and Enriching Methylome.....	197
10.3.1	Antibody-Based Recognition of Methylome.....	197
10.3.2	Recognizing Methylome with Kme Reader Domains.....	198
10.3.3	Recognition of Methylome Labeled with Clickable Chemical Tags.....	199
10.4	Choices of Processing Methylome Sample.....	199
10.4.1	Dissecting Methylome as Peptides or Full-Length Proteins.....	201
10.4.2	Choices of Proteolytic Reagents.....	202
10.4.3	Chemical Derivatization of Methylome for MS Analysis.....	202
10.5	Deconvolution of Methylome Sample.....	204
10.5.1	Protein Array to Reveal Methylome with Single-Target Resolution.....	205
10.5.2	Fractionation with Chromatography.....	205
10.6	Detection Methods for Methylome Profiling.....	206
10.6.1	Imaging-Based Detection Methods.....	206
10.6.2	MS-Based Detection Modules.....	207
10.7	Bioinformatics of Methylome.....	208
10.8	Examples of Integrative Modules for Methylome Profiling.....	209
10.8.1	Global Identification of Protein Lysine Methylation with Antibodies.....	209
10.8.2	Identification and Profiling of Protein Lysine Methylation with 3 × MBT Reader Domain.....	210

10.8.3 Profiling Substrates of EuHMT1 and EuHMT2 with BPPM.....	211
10.9 Perspective	211
Acknowledgments	212
References	212

CHAPTER 11 Bioinformatics and Biostatistics in Mining Epigenetic Disease

Markers and Targets..... 219

Junyan Lu, Hao Zhang, Liyi Zhang, and Cheng Luo

11.1 Introduction.....	219
11.2 Acquisition and Processing of High-Throughput Epigenomic Data.....	221
11.2.1 Bisulfite Sequencing.....	222
11.2.2 Microarray and ChIP-on-Chip.....	223
11.2.3 Next-Generation Sequencing.....	223
11.2.4 Mass Spectrometry	226
11.3 Bioinformatics and Biostatistics in Mining Epigenetic Biomarkers.....	226
11.4 Bioinformatics and Biostatistics in Mining Epigenetic Disease Targets.....	230
11.5 Useful Bioinformatic Resources	232
11.5.1 Databases	232
11.5.2 Software and Packages	235
11.6 Conclusions and Future Perspectives.....	237
References	240

CHAPTER 12 Computational Modeling to Elucidate Molecular Mechanisms of Epigenetic Memory

245

Jianhua Xing, Jin Yu, Hang Zhang, and Xiao-Jun Tian

12.1 Introduction.....	245
12.2 Identify Puzzle from Experimental Studies.....	247
12.3 Formulate Mathematical Model.....	248
12.4 Choose Appropriate Modeling Techniques	250
12.5 Determine Model Parameters.....	252
12.5.1 Nonspecific Background Free Energy of Binding of Enzymes	252
12.5.2 Free Energy of Binding of Enzymes within the Nucleation Region.....	254
12.5.3 Enzyme Lateral Interactions.....	254
12.5.4 Enzyme Rate Constants.....	254
12.5.5 Histone Exchange	254
12.6 Perform Computational Studies	255
12.7 Identify Insights from Model Studies and Make Testable Predictions.....	257
12.8 Conclusion	260
References	261

CHAPTER 13 DNA Methyltransferase Inhibitors for Cancer Therapy 265*José L. Medina-Franco, Jakyung Yoo, and Alfonso Dueñas*

13.1	Introduction.....	266
13.1.1	Epigenetics.....	266
13.1.2	DNA Hypomethylation in Cancer.....	266
13.1.3	DNA Hypermethylation and Gene Silencing in Cancer.....	267
13.1.4	DNA Methyltransferases	268
13.2	Development of DNMTi as Cancer Therapy	271
13.2.1	DNMTi in Clinical Use: Nucleoside Analogs	271
13.2.2	Nucleid Acid-Based.....	273
13.2.3	Non-Nucleoside Analogs.....	273
13.3	Advances in Experimental Methods	274
13.3.1	Assessment of DNA Methylation	274
13.3.2	Benchmarking of Methods for Methylome Analysis	275
13.3.3	Methods to Screen Compound Data Sets	275
13.4	Progress on the Identification of Novel DNMTi	276
13.4.1	Chemical Synthesis.....	276
13.4.2	High-Throughput Screening	277
13.4.3	Drug Repurposing.....	278
13.4.4	Natural Products and Dietary Sources	278
13.5	Advances in Computational Studies	280
13.5.1	Chemoinformatic Analysis.....	281
13.5.2	Molecular Docking of Small Molecules	282
13.5.3	Virtual Screening.....	283
13.6	Data Resources for DNMTi	283
13.7	Conclusions.....	284
	Acknowledgments	285
	References	285

CHAPTER 14 Histone Acetyltransferases: Enzymes, Assays, and Inhibitors 291*Yepeng Luan, Liza Ngo, Zhen Han, Xuejian Wang, Meihua Qu,
and Y. George Zheng*

14.1	Histone Acetyltransferases	292
14.2	The Pharmacologic Significance of HATs	294
14.3	HAT Biochemical Assays	296
14.3.1	Radiometric Assays	296
14.3.2	Coupled Spectrophotometric and Spectrofluorimetric Assays	298
14.3.3	Immunosorbent Assays.....	299
14.4	HAT Inhibitors	299
14.4.1	Bisubstrate HAT Inhibitors	300
14.4.2	Small Molecule HAT Modulators.....	302

14.5 Conclusion and Perspective	308
14.5.1 HAT Enzyme Discovery	308
14.5.2 Challenges in HAT Assays	309
14.5.3 Development of HAT Modulators	309
Acknowledgments	310
References	310
 CHAPTER 15 <i>In Vitro</i> Histone Deacetylase Activity Screening: Making a Case for Better Assays	319
<i>Quaovi H. Sodji, James R. Kornacki, Milan Mrksich, and Adegboyega K. Oyelere</i>	
15.1 Introduction.....	319
15.2 Overview of HDAC Assays	321
15.2.1 Sirtuin Activity Assays.....	321
15.2.2 Zinc-Dependent HDAC Activity Assays	324
15.3 Fluorogenic HDAC Activity Assay	325
15.4 Disadvantages of HDAC Fluorogenic Assays.....	325
15.5 HDAC Assay Using the Self-Assembled Mono-Layers for Matrix- Assisted Laser.....	326
15.5.1 Desorption Ionization Time-of-Flight Mass Spectrometry.....	326
15.6 Comparison of the Fluorogenic and SAMDI-MS HDAC Assays — Our Experience	326
15.7 Conclusion	330
References	330
 CHAPTER 16 Enzymatic Assays of Histone Methyltransferase Enzymes	333
<i>Hao Zeng and Wei Xu</i>	
16.1 Introduction.....	334
16.1.1 Histone Lysine Methyltransferases	334
16.1.2 Protein Arginine Methyltransferases.....	336
16.2 Enzymatic Assays for Histone Methyltransferases	337
16.2.1 Radioactive Assays.....	337
16.2.2 Fluorescence Polarization Assays	340
16.2.3 Microfluidic Capillary Electrophoresis	342
16.2.4 Fluorescence Lifetime Assay	343
16.2.5 Coupled Enzyme Assays	344
16.2.6 Antibody-Based Assays.....	347
16.2.7 Other Reporter Assays for Histone Methyltransferases	352
16.3 Conclusions.....	354
References	356

CHAPTER 17 Histone Methyltransferase Inhibitors for Cancer Therapy 363

*Keqin Kathy Li, Kenneth Huang, Shukkoor Kondengaden, Jonathan Wooten,
Hamed Reyhanfard, Zhang Qing, Bingxue Chris Zhai, and Peng George Wang*

17.1	Introduction.....	364
17.2	Histone Lysine Methyltransferases	365
17.2.1	DOT1L.....	365
17.2.2	Enhancer of Zeste (EZH2)	374
17.2.3	G9a.....	377
17.2.4	Mixed Lineage Leukemia.....	380
17.3	Protein Arginine Methyltransferases.....	383
17.3.1	PRMT Structure and Active Domain.....	383
17.3.2	PRMT Classifications.....	385
17.3.3	PRMTs as Therapeutic Targets for Diseases.....	387
17.3.4	PRMT Inhibitors.....	388
17.4	Conclusion	389
	References	390

CHAPTER 18 Discovery of Histone Demethylase Inhibitors 397

*Alexander-Thomas Hauser, Martin Roatsch, Johannes Schulz-Fincke,
Dina Robaa, Wolfgang Sippl, and Manfred Jung*

18.1	Introduction.....	398
18.2	Histone Demethylases and Their Involvement in Disease.....	398
18.2.1	JumonjiC Domain-Containing Histone Demethylases	398
18.2.2	Lysine-Specific Demethylase 1.....	399
18.3	Assays for Histone Demethylases and Their Role in the Discovery of Inhibitors	401
18.3.1	JumonjiC Domain-Containing Histone Demethylases	401
18.3.2	Lysine-Specific Demethylase 1.....	408
18.4	Structural Aspects of Inhibitor Binding.....	412
18.4.1	JumonjiC Domain-Containing Demethylases	412
18.4.2	Lysine-Specific Demethylase 1.....	415
18.5	Conclusion	418
	Acknowledgments	418
	References	418

**CHAPTER 19 Histone Demethylases: Background, Purification,
and Detection 425**

Kelly M. Biette, Joshua C. Black, and Johnathan R. Whetstine

19.1	Introduction.....	426
19.1.1	Historical Perspective	426

19.2	Purification of KDMs	428
19.2.1	Classic Bacterial Purification	428
19.2.2	Alternative Tags and Purification Approaches from Bacteria	429
19.2.3	Baculoviral Purification from Sf9 Cells	430
19.3	<i>In Vitro</i> Assays to Evaluate Demethylase Activity	431
19.3.1	Enzymatic Reactions	431
19.3.2	MALDI-TOF	432
19.3.3	Western Blot Analysis	435
19.3.4	Radiolabeled Formaldehyde Release Assay	435
19.3.5	ELISA-Based Assays	437
19.3.6	Fluorescent and Luminescent Assays	437
19.4	<i>In vivo</i> Detection of KDM Activity in Cell Culture Models	438
19.4.1	Analysis of KDM Activity by Immunofluorescence	439
19.4.2	Analysis of KDM Activity by Histone Purification and Western Blot	441
19.4.3	Analysis of KDM Activity by Chromatin Immunoprecipitation	441
19.5	Conclusion	442
	Acknowledgments	443
	References	443

CHAPTER 20 Animal Model Study of Epigenetic Inhibitors 447

Aili Chen and Gang Huang

20.1	Introduction	448
20.2	Epigenetic Inhibitors	449
20.2.1	Inhibitors of DNMTs	450
20.2.2	Inhibitors of HDAC	453
20.2.3	Inhibitor of HATs	454
20.2.4	Inhibitor of Histone Lysine Methyltransferases	455
20.2.5	Inhibitor of KDMTs	456
20.2.6	Inhibitor of PRMTs	457
20.2.7	Inhibitor of PRC1	458
20.2.8	Inhibitor of JAK2, IDH1/2	458
20.2.9	Inhibitor of the Bromodomain-Containing Family	459
20.2.10	Inhibitor of Splicosome	459
20.3	Use of Animal Model to Study the Inhibitors	460
20.3.1	Use of Animal Models to Study DNMTs (DAC and 5-Aza)	462
20.3.2	Use of Animal Models to Study Inhibitors of HDATs	463
20.3.3	Use of Animal Models to Study DOT1L Inhibitors	464
20.3.4	Use of Animal Models to Study EZH2 inhibitors	464
20.3.5	Use of Animal Models to Study Inhibitors of KDM Family and LSD Family	465
20.3.6	Use of Animal Models to Study IDH1/2 Inhibitors	466

20.3.7 Use of Animal Models to Study Epigenetic Inhibitors in Combined Therapy	467
20.4 Perspective and Conclusion	468
References	468
 Index	 479