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Preface

Target-based drug discovery continues to be a dominating paradigm in industrial research, and current strategies for epigenetic therapy are no exception. Already in 1942 Waddington introduced the term epigenetics [1]. He defined it as "the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being" [2]. Originally, epigenetics referred to all molecular pathways modulating the expression of a genotype into a particular phenotype. Later on, with the rapid growth of genetics, its meaning has gradually narrowed. Epigenetics has been defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence" [3, 4].

Epigenetic changes such as DNA methylation and histone methylation and acetylation alter gene expression at the level of transcription by upregulating, downregulating, or silencing genes completely. At the molecular level, epigenetic regulation involves hierarchical covalent modification of DNA and the proteins that package DNA, such as histones. Dysregulation of epigenetic events can be pathological, leading to cardio-vascular disease, neurological disorders, metabolic disorders, and cancer development, whereas the main focus of epigenetic drug discovery efforts has been on cancer. Thus, identifying drugs that inhibit these epigenetic changes are of great clinical interest [5].

Considering this prominent role in drug development, nine years ago a volume on "Epigenetic targets in drug discovery" [6] was published, covering in detail the available knowledge on methodology, epigenetic target classes, and inhibitor development. Since that period, novel findings in this field accumulated very fast. Thus, we felt time is mature to organize a second edition focusing on these recent developments. The editors of the initial book, Wolfgang Sippl and Manfred Jung, agreed to organize also the updating task. The new volume, presented here, focuses on medicinal chemistry applied to epigenetic targets, one of the fastest growing areas of drug discovery in recent years and comprises three parts. The introduction describes the gain of knowledge within the last decade. The second part concerns current methods including structural biology of epigenetic targets, computer-based technologies, mass spectrometry, peptide microarrays, chemical probe development, and epigenetic multi-targeting. The third part focuses on epigenetic targets like HDAC, SirT, HAT, methyltransferase and demethylase modulators, DNA modifiers, bromodomain and methyl-lysine reader proteins, and parasitic epigenetic targets.

We thank Wolfgang Sippl and Manfred Jung for organizing this volume and to work with such excellent authors. Last, but not least we thank Frank Weinreich and Waltraud Wüst from Wiley-VCH for their valuable contributions to this project and to the entire book series.

Raimund Mannhold
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May 2018

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Foreword

The role of histone becomes, thus, part of the problem of how the environment affects gene activity. Biology has by now outgrown the abstract and rigid limitations of classical genetics; for now it is clear that the chromosome, like other centres of vital activity, is subject to regulation by feed-back of the periphery.

A. E. Mirsky, 1965 (sic !) [1]

We had already used this quotation in the foreword for the first edition of this book in 2009. Still it is fascinating to read these prophetic words from more than 50 years ago. Already in 1950 Stedman had discussed the role of histones in differentiation [2] and in 1964 Allfrey reported on the acetylation on histones [3]. The words of Mirsky are from a Ciba Foundation symposium on histones and already then, a “functional correlation between histone acetylation and the RNA-synthetic capacity of the chromatin” was suggested.

Since the first edition of our book in 2009 the field of Epigenetics and the related drug discovery and development efforts have made amazing progress. By now, two inhibitors of DNA methyltransferases and five inhibitors of histone deacetylases (HDACs) have been approved for cancer treatment. Among the latter, tucidinostat (Chidamide) is of special interest as it is has been developed in China by a Chinese drug discovery company (Chipscreen Biosciences) [4] and we expect to see an increasing importance of China not only in science but also in drug development in the upcoming years. In addition, it is the first class-I selective orally available HDAC inhibitor approved and it will be interesting to see if resp. for which patients the class selectivity will have an advantage.

For other targets, like histone methyltransferase and demethylases, the therapeutic potential was already clearly visible nine years ago but now several of them have indeed moved into clinical trials [5] and the results of these are awaited with great anticipation. For acetyltransferases, the progress is still very limited but just recently the first clearly drug like HAT inhibitor has been presented [6] and it will be interesting to see if this “oldest class” of epigenetic target will still find its way to patients.

One target class that had not been covered in our first edition of 2009 are the so-called reader proteins that specifically bind to acetylated resp. methylated lysines and mediate the signals that have been set and maintained by the

equilibrium of acetyltransferases and deacetylase resp. methyltransferase and demethylases. The inhibitors of the acetyl-lysine readers (bromodomain containing proteins) have developed at an amazing pace from the groundbreaking publication in 2010 [7] to several candidates in clinical trials already. The methyl lysine readers still lag a bit behind in their development but first inhibitors have emerged and there is also a perspective to target recognition of non-histone client proteins for these readers [8].

The technique of chemically induced proteolysis (PROTAC approach) [9] has received specific interest due to its recent application in the field of epigenetics [10] and shows great potential as an approach that is conceptually different from standard chemical target inhibition. Thus, we can expect more exciting years for biology and drug discovery and development in the field of epigenetics.

We apologize to all scientists whose efforts in the field were not duly cited in this book. We thank our authors, the editors and publishers from Wiley-VCH and our families for support.

Wolfgang Sippl
Manfred Jung

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Part I

Introduction – Epigenetics

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Epigenetics: Moving Forward

Lucia Altucci

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Both the focus on epigenetics and the simple use of the term "epigenetic" have significantly augmented since the 1940s, when Sir Conrad Waddington opened the ground to this field. Since then, the definition of epigenetics became more inclusive, often defined as "*stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence*" (2008 Cold Spring Harbor Epigenetics meeting). In more common words, the term epigenetics derives from επί-genetics, which literally means "on top of" genetics, referring to the modifications of chromatin that are able to switch genes "on" or "off" affecting the cell's "interpretation" of genes and consequently function, specialization, phenotype, and cell fate [1].

Recently, the importance of the epigenetics has become evident from the plethora of articles, conferences, and consortia on the topic over the past decade. All over the world, research was intensified more and more on basic as well as biomedical-oriented epigenetic-based methodologies, targets, and applications. Funders initiated concerted actions to promote standardization and collaboration of the worldwide efforts aiming to unveil the role of transcriptional and epigenetic mechanisms in specification of cell fates and functions, such as the "American Association for Cancer Research Human Epigenome Task Force" and the "European Union Network of Excellence." The "International Human Epigenome Consortium" (IHEC) was founded to coordinate and standardize the production of reference epigenomes with a focus on cell states relevant to health and diseases, thereby accelerating translation of new knowledge to improve therapy [2]. IHEC has also coordinated the international efforts by bringing together the European Commission that funded "Blueprint consortium" (http://www.blueprint-epigenome.eu/) with, as a mere example, the NIH that funded "Roadmap on Epigenomics" (http://www.roadmapepigenomics.org). In addition, IHEC introduced common bioinformatics standards, models, and tools to analyze and interpret epigenomic data in a uniform and interoperable manner [3].

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1.1 Why This Enormously Increased Interest?

One reason is the need to address fundamental questions to understand the way the genome and environment interact in development and aging and how the epigenome affects or is affected by health and disease.

In addition, there is an urgent need to develop new ways to "drug" the epigenome and to translate discoveries into improvements of human health. Despite being quite stable and hereditable, epigenome modifications can be easily changed within the cell, affecting cell fate and functions. This epigenome plasticity opens the way to the pharmacological exploitation and to the identification and characterization of chromatin-targeting drugs. The identification of increasing numbers of new players acting as "*writers, erasers, or readers*" of the epigenome suggests that an intricate and very well-defined epi-modulated setting is responsible for maintaining the plasticity potential, ultimately guarantying cell identity and cell heterogeneity of otherwise similar tissues. Given that new modifications/new players are being uncovered, additional complexity arises, and a better understanding and frequent revisiting of the mechanism(s) of chromatin regulation and plasticity – ultimately at the single-cell level – are needed. The potential of this emerging knowledge toward its translation into biomedical applications is breathtaking. For example, a huge number of studies (many of which using high-throughput approaches) have unveiled the significance of certain histone marks, epi-enzymes, and chromatin-regulating factors in different human pathologies such as cancer, neurological disorders, diabetes, immunological pathologies, etc. [4]. Translating this basic knowledge to bedside practice has triggered investments in the identification and development of new drugs able to re-equilibrate deregulated epigenome areas acting by inhibiting or (currently more rarely) activating chromatin enzymes and/or by interfering the function of chromatin readers.

In addition to the rapidly accumulating knowledge on the mechanisms of action of chromatin-targeting "(epi)drugs," we have only beginning to unravel the different substrates of the epi-enzymes. "Epi-drugs" are designed to inhibit (or activate) histone-modifying enzymes or DNA methyltransferases or to interfere with readers of the resulting chromatin modifications. However, these chromatin modifiers (and the respective "epi-drugs") affect various substrates, including proteins in signal transduction pathways and cell structure. Such insights will turn out to be crucial to develop a better rational design of drugs treatment (and combination thereof), further exploiting and expanding the promise of epigenetically acting drugs.

It is still debated whether selective or broad chromatin modulators will be more effective [5]. As has been demonstrated in some cancer types harboring mutated enzymes, a selective "epi-drug" approach (active exclusively or preferentially on the mutant) may be preferred. On the other hand, a broad modulator might become more useful when concomitant alterations of different epi-targets are playing a role. This might also include hybrid molecules acting contextually on one epi-target and one non-epi-target.

Among the best studied chromatin-targeting drugs, HDAC inhibitors [6] and DNA-demethylating agents [7] have entered the clinic for anticancer treatment

and prevention. Despite that HDAC inhibitors mostly induce hyperacetylation, this cannot be considered as a parameter of response. This issue highlights the need for a detailed understanding and development of markers of treatment response along with (epi)drug development. This will become a challenging task considering that epigenetic-based approaches have been proposed for very different diseases. In cancer patients, the altered expression of epi-players (overexpression or silencing) or a qualitative deregulation such as the mutation in one of the epi-enzymes has been one of the parameters of choice although patient's stratification on the basis of HDAC expression levels appears not always predictive of a better response. The presence of a well-characterized target mutation may instead prove to be more useful for patient stratification. Small molecules able to selectively modulate the mutated enzymes/targets may display tumor-specific action.

Interestingly, different groups of enzymes display diverse ways of deregulation; for example, HDACs are generally quantitatively overexpressed in cancer [8] (with the exception of HDAC2 mutations [9], for example, in colon tumorigenesis), whereas HATs appears more frequently mutated [10, 11]. Furthermore, the direct and indirect deregulation of methylation control through mutations in DNA methyltransferases and isocitrate dehydrogenases (IDH) genes appears to go along with abnormal histone and DNA methylation as a common feature of tumors with IDH1 and IDH2 mutations and altered stem cell differentiation and eventual tumorigenesis [12]. Description of inactivating mutations in TET2 suggests that cellular transformation is in part caused by the deregulation of 5-mC conversion. The TET enzymes have particular relevance in hematological cancers and solid tumors with mutations causing TET inactivation [13].

1.2 Looking Forward to New Avenues of Epigenetics

The constant flow of discoveries in the epigenetic field adds new layers of complexity and may lead to novel approaches for treatment. Novel chromatin marks are identified, and insight from mining of these targets (alone and within the context of others) may rapidly change our view. For example, hydroxymethyl cytosine and its modulation is at present a focus of discussions aimed at unraveling its mode of action and its potential role in cancer as well as other human diseases [14] [15]. The levels of 5hmC in the brain of patients with neurodegenerative disorders have been reported to be highly compromised, indicating a potential role of 5hmC in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease. It has yet to be established whether this is the cause or the consequence of the onset and progression of these diseases [16].

The burst in acquisition of scientific knowledge and in evolving new technologies will also pave the way to new concepts in the regulation and deregulation of the epigenome. Emerging single-cell epigenomic methods [17] are being developed with the exciting potential to transform our knowledge of gene regulation [18]. Until recently, our epigenetic modifications have been studied in bulk measurements in populations of cells.

The development of single-cell technologies is likely to cause a profound transformation of epigenome studies and their interpretation, in particular, in cases where (epi)genetic heterogeneity is overriding. In recent years, many of the high-throughput sequencing technologies hither to assaying population have been adapted and became assayable at the single-cell level. Combined single-cell methods such as simultaneous assessment of the transcriptome and DNA methylome may provide deeper insight in epigenetic–transcriptional correlations, allowing analyses on the causal relationships between phenome and the epigenome state. Furthermore, combined genome and epigenome analyses will likely open up new avenues to dissect the complex contribution of genomic and epigenomic heterogeneities [19].

A better integration of high-throughput data, bioinformatics interpretation, novel epi-marks, and chromatin players has the potential to bridge basic knowledge with the clinics both for epi-marks mining for diagnosis of disease treatment and outcome prediction and for disease prevention. Furthermore, many chromatin-targeting drugs have been identified and characterized in the past decade for their beneficial action against different human diseases. Even though the beneficial effect and link to the selective chromatin-regulating action has to be better corroborated and strengthened, their clinical potential is clear. In agreement, HDACi have been approved for the therapy of cutaneous T-cell lymphoma (CTCL) and recently for the treatment of multiple myeloma [20], as are DNA-demethylating agents for the treatment of myelodysplastic syndrome (MDS). In addition, the action of HDAC inhibitors against cancer might also be linked to the modulation on the immune system, potentially shedding a different light for their clinical use [21]. That histone methylation is also altered in cancer that led to the identification of lysine methyltransferases and demethylases as promising targets for new anticancer drugs. Inhibitors (targeting the histone methyltransferases DOT1L and EZH2 as well as the demethylase LSD1) have already reached the first stages of clinical trials in cancer therapy [22].

Also pharmacological inhibition of BET proteins shows therapeutic action in a variety of different pathologies, particularly in models of cancer and inflammation [23]. Such effects have been attributed to subsets of downstream targets. While it is clear that the therapeutic potential is huge, the current understanding of molecular mechanisms that underlie the therapeutic effects of pharmacological BET bromodomain inhibition still need better understanding [24].

Drug discovery efforts in the epigenetic field are not only focused on cancer but also on more chronic diseases opening the way to new opportunities for the epi-targeted treatments. For example, I-BET151 has been reported to effectively prevent type 1 diabetes in a mouse model for this disease [25, 26], suggesting that an epigenetic treatment of diabetes might be at our doorstep. Along these lines, different classes of “epi-drugs” that have been suggested to decrease obesity and clinical trials at different stages are ongoing, aiming to a better definition of their potential [27]. Recent studies have identified SIRT1 activators that may delay multiple diseases of aging and extend lifespan *in vivo* [28]. In theory, such molecules could act against diseases, potentially extending healthy years of life. Potential roles of SIRT1 and SIRT2 modulation in neurodegenerative diseases

have been proposed [29, 30] and an SIRT1 inhibitor (Selisistat) is in clinical trial against Huntington's disease [31].

These are only examples of the critical need to illuminate the drug discovery efforts in the identification and characterization of the novel epi-drugs [32]. Thus, in this volume an overview of state-of-the-art knowledge and development in drug design for epi-targets, their mechanisms of actions, and the increasing spectrum of applications is presented. Furthermore, current methodologies are discussed including the structural biology of epigenetic targets, computer-based technologies, mass spectrometry, peptide microarrays, chemical probe development, and epigenetic multi-targeting. In addition, the "epi-drug" classes such as HDAC, SirT, HAT, methyltransferase and demethylase modulators, DNA modifiers, bromodomain, and methyl-lysine reader proteins are examined. Finally this volume will also address challenges and promises of parasitic epigenetic targets. A new promising approach is chemically induced proteolysis by so-called PROTACs (proteolysis targeting chimeras), where a ligand to the target of interest is fused to a moiety that leads, e.g. to ubiquitinylation and subsequent proteolytic degradation. This will phenocopy knockdowns, resp. knockout studies, and is promising prolonged target inactivation and might become a new paradigm in drug discovery and hence also in epigenetics [33–35].

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Part II

General Aspects/Methodologies

2

Structural Biology of Epigenetic Targets: Exploiting Complexity

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2.1 Introduction

In the last two decades, epigenetic effectors have increasingly been shown to be major regulators of nuclear processes, with direct implications for cell homeostasis, response to external stimuli, development, and onset and progression of many diseases [1]. As a consequence, both fundamental research in epigenetics and the development of epigenetic drugs (epi-drugs) for therapy have become major fields of investigation.

Initial studies focused on epigenetic enzymes involved in the deposition and removal of epigenetic marks and on the reader domains responsible for the specific recognition of these marks [1, 2]. Yet, the discovery that other epigenetic effectors such as histone variants, histone chaperones, and ATP-dependent chromatin remodelers are also implicated in diseases further broadens the number of targets for epi-drug design [1, 3].

A few epi-drugs are already approved for the treatment of diseases, notably cancer [2c, 3c]. Their clinical use is often accompanied by serious undesirable side effects due to the fact that many epigenetic effectors belong to families whose members are often functionally different but structurally similar. This makes selective inhibition a major issue for the design of next-generation epi-drugs. In this respect, structural information is invaluable in helping deciphering precisely in molecular terms the mechanisms governing epigenetic processes and in aiding next-generation epi-drug design.

Another important reason for the reduced usage of epi-drugs is the strong interplay between epigenetic effectors. Notably, many epigenetic effectors act within large macromolecular complexes that represent the bona fide functional epigenetic units and that bear different epigenetic activities. This organization has two major consequences for the design and the use of epi-drugs. First, these complexes are physically and functionally linking epigenetic activities. Thus, modulating one activity with small molecules is likely to affect the other activities. Second, regulatory subunits can change partner/substrate recognition, enzymatic activity/kinetics, and inhibitor binding. Here again, deciphering the

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structures of these large molecular assemblies, or at least those of their active sub-complexes, is of paramount importance for understanding epigenetic mechanisms and for aiding epi-drug design.

A wealth of structural data has already been obtained on epigenetic effectors and their interactions with inhibitors, substrates, and protein partners, unraveling the diversity and complexity of these interactions. The huge amount of published structural data prevents an exhaustive description of all these results. Chapter 2 on epigenetic enzymes [2b] and specific chapters of this book are providing precise structural information on epigenetic enzymes and readers. In this chapter, we have chosen to focus primarily on epigenetic macromolecular complexes from the various classes of epigenetic effectors whose structures have enlarged our understanding of epigenetic mechanisms and pave the way for designing next-generation selective epi-drugs. Specifically, macromolecular interactions as well as mechanisms leading to structural rearrangements are described, highlighting ways of modulating the activity of epigenetic effectors.

2.2 DNA Methylases: The DNMT3A–DNMT3L–H3 and DNMT1–USP7 Complexes

In human, DNA methylation occurs predominantly on cytosines (5-methylcytosine) in CpG motifs that often form clusters known as CpG islands [4]. The initial view that DNA methylation is a rather stable epigenetic mark has been completely revisited in the last decade as new demethylation pathways have been characterized [5]. It is now commonly accepted that DNA methylation is a highly dynamic mark that is important in developmental processes. Specifically, methylation patterns are strongly perturbed in diseases, notably in cancers [4, 5].

De novo DNA methylation is carried out by the DNA methyltransferases DNMT3A and DNMT3B, whereas DNMT1 is required for the maintenance of the methyl mark by methylating hemimethylated DNA. DNMT3L, an inactive paralogue of DNMT3A/B, binds to and stimulates the activity of DNMT3A. DNMT3A activity is also stimulated in a DNMT3L-independent manner by histone H3 when its lysine 4 is not methylated. DNMT3A and DNMT3L both have an ADD (ATRX-DNMT3-DNMT3L) domain followed by a methyltransferase domain. Yet, DNMT3A catalytic domain (CD) is active, whereas the one of DNMT3L (CD-like) is inactive.

The ADD domains of DNMT3A and DNMT3L are able to bind to the N-terminus of histone H3 when it is not methylated on lysine 4. Methylation prevents binding due to steric hindrance [6]. The 3.8 Å resolution crystal structure of DNMT3A (ADD-CD) bound to DNMT3L (CD-like) shows that DNMT3L (CD-like) forms an extensive interaction with DNMT3A CD. This suggests that stimulation of DNMT3A activity by DNMT3L comes from a stabilization of the DNMT3A CD (Figure 2.1a) [7]. However, DNMT3A ADD domain and the linker region that connects it to DNMT3A (CD) pack against DNMT3A CD at a position where substrate DNA would be expected to bind, indicating

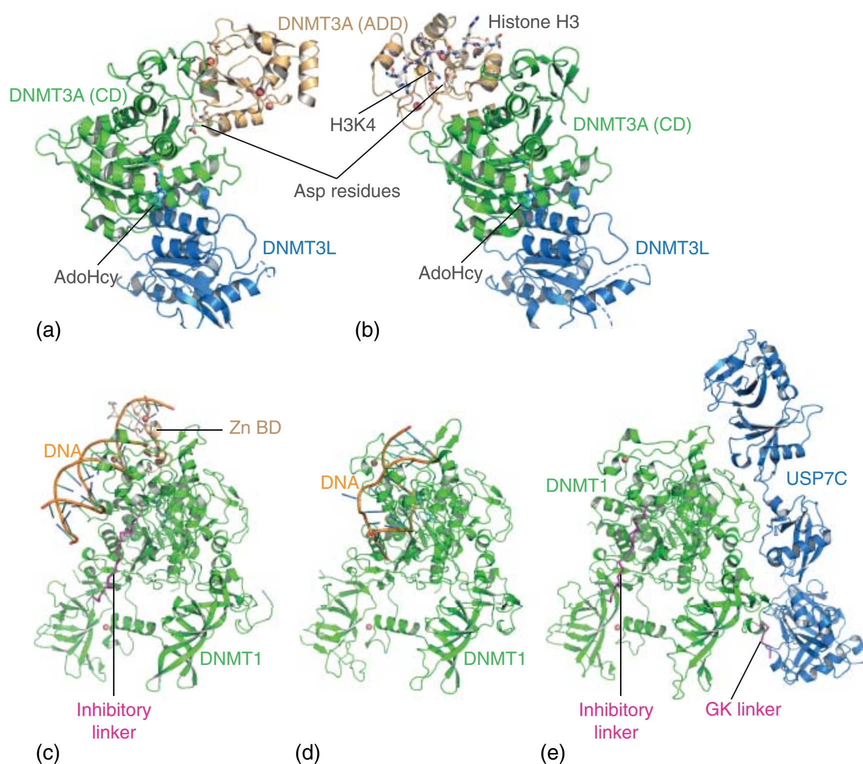

Figure 2.1 Structures of DNA methyltransferases. Ribbon representation of DNA methyltransferases (a, b) Structures of the *de novo* DNA methylation complex DNMT3A–DNMT3L in inhibitory (a) and activated (b) states. DNMT3A catalytic domain (CD) is colored green and its ADD (ATRX–DNMT3–DNMT3L) domain is colored wheat. DNMT3L inactive CD is colored blue. Zinc ions are shown as red spheres. Histone H3-activating peptide and important DNMT3A aspartate residues are represented as sticks with gray carbons. An AdoHcy (S-adenosyl-L-homocysteine) molecule is represented as sticks with cyan carbons. The coloring is identical in all figures unless stated. (c–e) Structures of methyl mark maintenance DNA methyltransferase DNMT1 in inhibitory (a) and active (b) states and in a stabilizing complex with USP7 (c). DNMT1 is shown in green, DNA in orange, and USP7 in blue. DNMT1 zinc binding domain (Zn BD; wheat) recognizing unmethylated DNA is shown as well as the inhibitory and regulatory GK linkers (magenta ribbons).

that this structure represents an inhibitory form of the DNMT3A–DNMT3L complex.

Upon binding of histone H3 N-terminus unmethylated on lysine K4 to DNMT3A ADD domain, the ADD domain makes a large movement, interacting with another surface of DNMT3A (CD), thus freeing the DNA binding surface of this CD (Figure 2.1b) [7]. Specifically, H3K4 binds to DNMT3A ADD domain aspartate residues that are otherwise involved in the formation of the inactive DNMT3A conformation.

DNMT1 is also an essential DNA methylase and is the target of two of the few FDA-approved epi-drugs [2c, 3c]. In contrast to DNMT3A/B enzymes, DNMT1 can only methylate hemimethylated DNA. The 3.0 and 2.6 Å crystal structures of

DNMT1 bound to non-methylated DNA and to hemimethylated DNA suggest a mechanism by which this enzyme carries out this discrimination [8]. Specifically, unmethylated DNA is recognized by a zinc finger of DNMT1 [8a]. This recognition positions the linker that connects the zinc finger to the first bromo-adjacent homology 1 (BAH1) domain of DNMT1 between the DNA and the active site of the enzyme, leading to an inactive complex (Figure 2.1c).

In the structure of the productive complex [8b], the DNA is found inserted into the active site of the CD (Figure 2.1d). Actually, in this structure the major conformational change observed concerns the catalytic loop that adopts a conformation compatible with catalysis. Yet, this structure was obtained with a shorter construct of DNMT1 that does not encompass the zinc finger and the following linker that are playing a major role in DNMT1 autoinhibition in the presence of unmethylated DNA. It remains therefore to be understood whether the presence of hemimethylated DNA prevents zinc finger binding and autoinhibition or whether the removal of the inhibition is due to an active mechanism.

DNMT1 has been shown to be regulated through various pathways and partner proteins. One of them is the ubiquitin-specific protease 7 (USP7) that stabilizes DNMT1. The 2.9 Å crystal structure of USP7 C-terminus (USP7C) in complex with DNMT1 has been solved [9]. The overall structure of DNMT1 in this complex is highly similar to the one in the autoinhibited form, including the positioning of the DNMT1 inhibitory N-terminal linker in DNMT1 DNA binding site (Figure 2.1e).

The DNMT1–USP7C complex structure reveals that USP7C, which is composed of several ubiquitin-like domains, binds to DNMT1 on the side opposite to the methylase active site. A critical interaction is made with DNMT1 KG linker that contains several Lysine–Glycine repeats. Specifically, the lysines of this linker are forming multiple interactions with residues of USP7C, and acetylation of these lysines precludes interaction between USP7 and DNMT1, favoring the *in vivo* degradation of DNMT1 [9].

2.3 Histone Arginine Methyltransferases: The PRMT5–MEP50 Complex

Protein arginine methyltransferases (PRMTs) are monomethylating and symmetrically or asymmetrically dimethylating arginine residues in histones and other cellular effectors [10]. The role and mode of action of PRMTs have long remained poorly understood. This picture is however changing as more data is obtained on this class of enzymes, showing that they are also involved in a wide range of diseases. Specifically, to develop therapeutic strategies targeting these enzymes, the deciphering in molecular terms of the specific recognition by PRMTs of their substrates and of the influence of partner proteins on PRMTs activity and substrate recognition has to be addressed.

The 2.0 and 3.0 Å crystal structures of human and *Xenopus laevis* PRMT5 in complex with one of its partners, MEP50, have provided novel information on these issues [11]. PRMT5 monomethylates and symmetrically dimethylates

different substrates [10]. PRMT5 is composed of two domains: an N-terminal TIM barrel and a C-terminal CD that adopts a canonical arginine methyltransferase fold. The structures of the PRMT5–MEP50 complex reveal the formation of a tetramer of PRMT5–MEP50 dimers where PRMT5 forms the core of the octamer and MEP50 is located on the outside of the complex.

The 2.0 Å crystal structure of the human PRMT5–MEP50 complex in the presence of a AdoMet analogue and an H4 N-terminal tail peptide shows how the substrate is recognized in the active site of PRMT5 and suggests how active site residues participate to the methylation process [11a] (Figure 2.2a). Interestingly, the crystal structures of PRMT5–MEP50 bound to selective PRMT5 inhibitors show how these inhibitors can bind directly to these active site residues, leading to selective inhibition [12]. In addition, these different structures also reveals the molecular basis by which phosphorylation of tyrosine residues in the substrate binding groove can diminish catalytic activity by opposing to substrate binding.

Yet, these structures do not reveal the role of MEP50 in the complex. This information is provided by a lower resolution electron microscopy (EM) structure of the PRMT5–MEP50 complex bound to one of its substrate, nucleoplasmin. This structure reveals that nucleoplasmin interacts predominantly with MEP50 that serves as a docking platform for the substrate to be presented to PRMT5 [11b].

2.4 Histone Lysine Methyltransferases: The MLL3–RBBP5–ASH2L and the PRC2 Complexes

Proteins of the MLL family play major roles in development and are mainly responsible for the methylation of lysine 4 of H3 (H3K4), an epigenetic mark associated with activation of transcription [13]. MLL1 has been most studied

Figure 2.2 Structures of arginine and lysine methyltransferases. Ribbon representation of arginine and lysine methyltransferases. (a). Structure of the PRMT5–MEP50 dimer. PRMT5 arginine methyltransferase catalytic domain (CD) is colored green, its TIM-barrel domain is colored wheat, and MEP50 is colored blue. Histone H4 peptide binding to PRMT5 CD is shown as sticks with gray carbon as well as PRMT5 important active site residues and regulatory tyrosines. An AdoMet (S-adenosyl-L-methionine) analogue bound to PRMT5 CD is shown as sticks with cyan carbons. (b). Structure of lysine methyltransferase MLL3 (SET domain; green) in complex with a RBBP5 peptide (yellow) and ASH2L C-terminal domain (blue). Histone H3 N-terminal peptide and an AdoHcy molecule are shown as sticks with gray and cyan carbons, respectively. Residues at the interface of the three proteins that form a hydrogen bond network (MLL3 Arg network) are also shown as sticks. (c). Structure of the polycomb repressive complex 2 (PRC2) (EZH2/EED/SUZ12). Methyltransferase EZH2 is colored green except its catalytic SET domain that is colored wheat. EED is colored blue and the SUZ12 VEFS domain is colored yellow. An AdoHcy molecule bound to EZH2 SET domain is shown as sticks with cyan ribbons. Two H3 N-terminal peptides are shown as sticks with gray carbon: a H3K27me3 peptide bound to EED and a mutated H3M27 peptide bound to EZH2 SET domain. Two regulatory elements are shown in magenta: the SET activation loop (SAL) (shown as ribbon) and the stimulation-responsive motif (SRM). This latter motif is suggested to transmit the signal of H3K27me3 binding to the EED subunit to the catalytic SET domain.