Epigenomics

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In eukaryotes, the genomic DNA is tightly compacted into chromatin, the structure of which plays essential roles in genome function and gene expression [1]. The primary unit of chromatin is the nucleosome. The nucleosome core particle is comprised of histone H2A, H2B, H3, and H4, and is wrapped around by a segment of 147 bp DNA. Chromatin modifications include DNA methylation and histone modifications. DNA methylation in eukaryotes consists of the addition of a methyl group at position five of the pyrimidine ring of cytosine [2]. Histone modifications include acetylation, methylation, phosphorylation, and monoubiquitination, etc. DNA methylation and histone modifications are reversible and are recognized and bound by different chromatin protein complexes that usually have chromatin remodeling activities to alter chromatin structure [3, 4]. Chromatin modification profiles define distinct epigenomes which are reflected by specific gene expression patterns of different cell types and/or responses to variable environmental conditions. Epigenetic regulations involving variation of DNA methylation and histone modifications and histone variant deposition, etc., control transcriptional activity of genes, repetitive sequences and transposable elements, as well as DNA replication and repair [5]. In this chapter, we will describe recent advances in studies of rice chromatin modification, regulation and recognition mechanisms, and their function in controlling rice gene expression and plant growth.

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1 DNA Methylation in Rice

DNA methylation is a hall mark of epigenetic inactivation of repetitive sequences and transposable elements and heterochromatin formation in plants. In plant genomes, cytosine methylation occurs in CG, CHG, and CHH contexts (where H is A, C, or T). DNA methylation is highest within pericentromeric regions that are enriched for transposable elements and repetitive sequences including ribosomal DNA (rDNA). However a significant proportion of genes (15–20 %) also contain methylated cytosines in *Arabidopsis* and rice [6–9]. Methylation of CG sequences is commonly found within gene bodies, whereas methylation of non-CG (CHG and CHH) sequences is enriched in transposons and repetitive sequences. In genes, DNA methylation is distributed over the transcribed regions or gene bodies but in most cases is depleted from the 5′ and 3′ ends of the genes (Fig. 9.1). It is suggested that moderately

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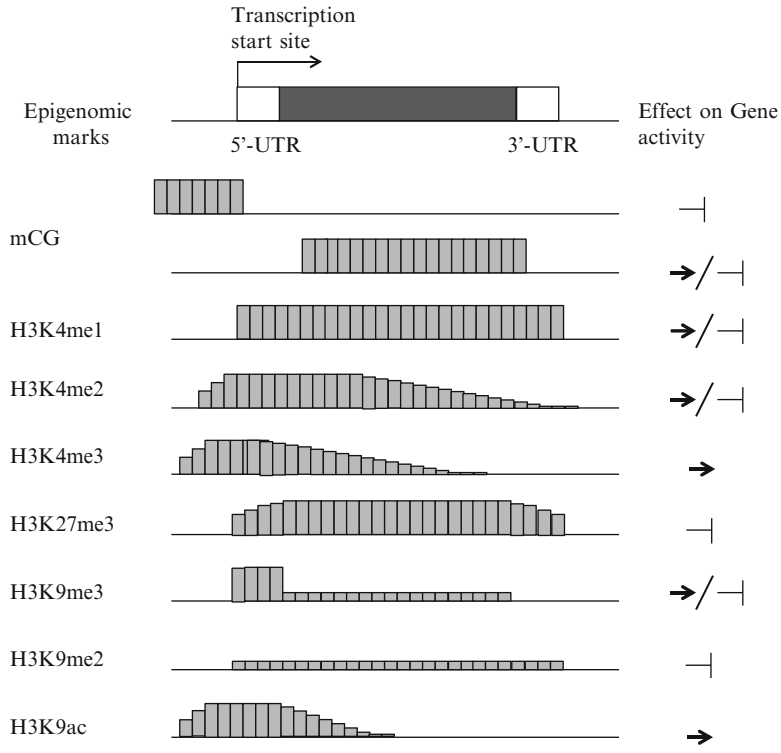

Fig. 9.1 Correlation of epigenomic modifications and gene activity. Distribution of indicated marks along the gene is shown. *Arrows*: transcriptional activation, *bars*: repression

expressed genes are more likely to be methylated than those with low or high expression [10]. Gene body methylation (i.e., CG methylation) is conserved between plants and animals and is hypothesized to suppress spurious initiation of transcription within active genes [7, 11]. About 5 % genes show DNA methylation within promoter regions, which has a repressive function on promoter activity. DNA methylation can inhibit transcription or lead to silent chromatin either by physically impeding the binding of transcription factors to the promoter or by interacting with methylcytosine-binding proteins that can recruit additional chromatin proteins to the locus to modify histones or remodel the chromatin thereby forming compact heterochromatin.

In *Arabidopsis*, CG methylation is maintained by the DNMT1 (DNA MethylTransferase 1) homologue, MET1 (Methyltransferase1), CHG methylation primarily by the plant-specific DNA methyltransferase CMT3 (ChroMomeThylase 3), and CHH methylation by DMR2 (Domains Rearranged Methyltransferase 2), a homologue of mammalian DNMT3 [12] (Fig. 9.2). In addition, DRM2 is responsible for de novo methylation of all three sequence contexts, which is directed by small interfering RNAs (siRNAs) called RNA-dependent DNA methylation (RdDM) [13]. While a general concept is that distinct DNA methyltransferases are responsible for either maintenance or de novo methylation in different sequence contexts, an emerging view is that different enzymes may cooperate to catalyze both steps. Rice genes encoding putative DNA methyltransferases and the siRNA machinery have been identified (Table 9.1). Loss-of-function mutations of rice DNA methyltransferase genes lead to reduction of DNA methylation of repetitive sequences (unpublished). DNA methylation can be lost by passive (non-maintenance during DNA replications) and active (enzymatic removal) mechanisms. *Arabidopsis* DNA demethylase DME (Demeter) and ROS1 (Repressor Of Silencing 1) have combined DNA

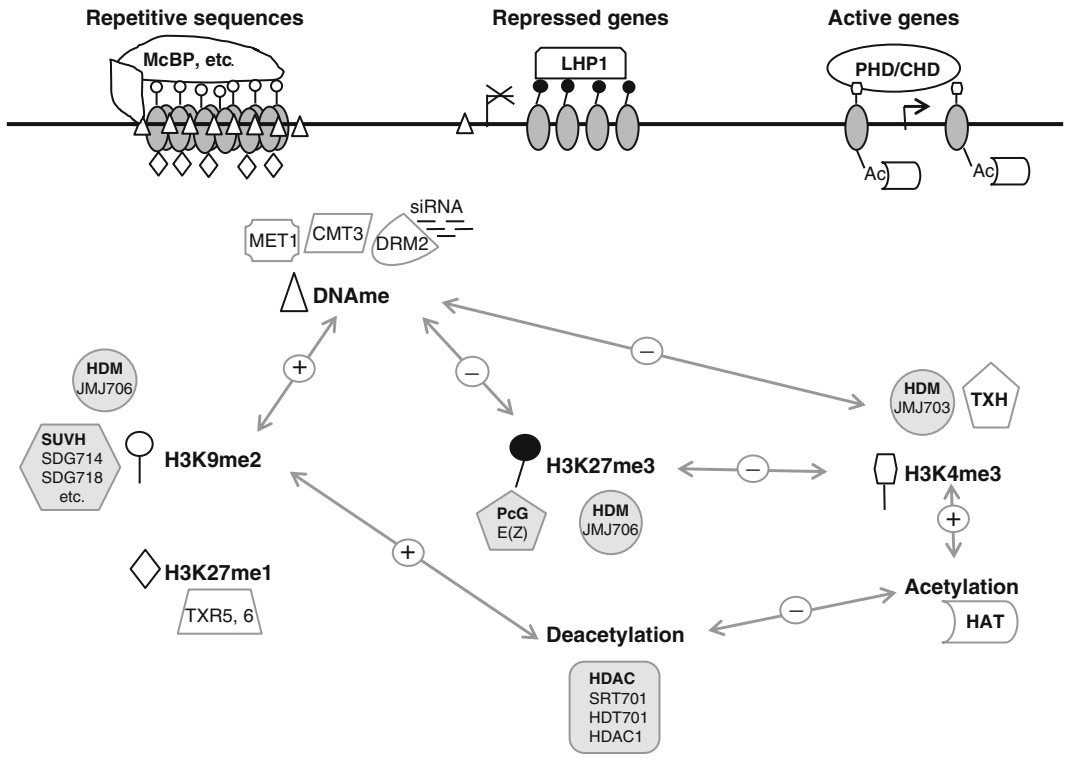

Fig. 9.2 Schematic representation of chromatin structures over repetitive sequence, repressed, and active genic regions. In repetitive sequence regions, cytosines are methylated at CG, CHG, and CHH sequence contexts. H3K9me2 and H3K27me1 are enriched. These modification marks are bound by proteins associated with heterochromatin such as McBP (methylated Cystosine Binding Proteins, etc.) and nucleosomes are highly condensed. In repressed genic regions histones are deacetylated and H3K27me3 is enriched which is bound by LHP1. In active genic regions, histone acetylation level is high and H3K4me3 is enriched at 5′ end of genes. Acetylated histone lysines are recognized by bromodomain-containing proteins (such as HAT) that have transcription coactivator function. H3K4me3 can be recognized by PHD or CHD proteins that facilitate transcription by RNA polymerase II. In plants, DNA methylation (DNAme) is catalyzed by three types of enzymes: MET1, CMT3 and DRM2. De novo DNA methylation mediated by DRM2 is trigged by siRNA. DNAme and H3K9me2 that is regulated by the SUVH (Su(var) homologue) class of histone methyltransferases (HMT, i.e., SDG714/718 in rice) and histone demethylase (HDM, i.e., JMJ706 in rice) are mutually agonistic. H3K27me3 is mediated by the E(Z) type of HMT which is the key component of the polycomb group (PcG) complexes and demethylated by HDM (probably JMJ706 in rice). H3K27me3 is antagonistic to H3K4me3 and DNAme. H3K4me3 is catalyzed by the trithorax homologue (TXH) proteins and demethylated by JMJ703 in rice. H3K4me3 inhibits DNAme and H3K27me3. Histone (mostly H3 and H4) lysines are acetylated by HAT (histone acetyltransferases) and deacetylated by HDAC (histone deacetylases, such as SRT701, HDAC1, and HDT701 in rice). Histone acetylation facilitates H3K4me3, while histone deacetylation facilitates H3K9me2. H3K27me1 is catalyzed primarily by TXH-related (TXR5, 6) proteins

glycosylase and AP lyase activities [14, 15]. The rice DNG701 protein that is closely related to *Arabidopsis* ROS1 has been shown to display 5-methylcytosine DNA glycosylase and lyase activities in vitro [16]. Knockout or knockdown of *DNG701* in rice leads to DNA hypermethylation and reduced expression of the retrotransposon *Tos17* [16].

2 Histone Modifications in Rice

2.1 Histone Acetylation/ Deacetylation

Strong acetylation of histones induces relaxation of chromatin structure and is associated with transcriptional activation, whereas weak or no

Table 9.1 Rice chromatin modification and remodeling genes

	Class	ChromDB name	Tigr locus		Class	ChromDB name	Tigr locus
DNA methyltransferase	MET1	DMT702	Os03g58400	SiRNA-related	Dicer-like	DCL701 [84]	Os03g02970
		DMT707	Os07g08500			DCL702	Os01g68120
	DRM1/2	DMT705	Os01g42630			DCL703 [85]	Os10g34430
		DMT706	Os03g02010			DCL704 [85, 86]	Os04g43050
		DMT708	Os12g01800			DCL705 [87]	Os03g38740
		DMT709	Os11g01810				Os09g14610
		DMT710	Os05g04330		Argonaute	AGO701	Os02g58490
	CMT3	DMT701	Os03g12570			AGO702	Os06g39640
		DMT703	Os05g13790			AGO703	Os01g16870
		DMT704	Os10g01570			AGO704	Os07g28850
DNA demethylase		DNG701	Os05g37350			AGO705	Os04g06770
		DNG702	Os01g11900			AGO706	Os03g58600
		DNG703	Os02g29230			AGO707	Os06g51310
		DNG704	Os05g37410			AGO708	Os04g47870
Methyl binding domain protein		MBD701	Os09g32090			AGO709	Os04g52540
		MBD703	Os06g48870			AGO710	Os03g47830
		MBD704	Os04g20560			AGO711	Os02g45070
		MBD705	Os04g19690			AGO712	Os07g09020
		MBD706	Os05g33550			AGO713	Os04g52550
		MBD707	Os12g42550			AGO714	Os03g33650
		MBD708	Os09g29750			AGO715	Os07g16230
		MBD709	Os08g37920			AGO716	Os02g07310
		MBD710	Os02g09920			AGO717	Os03g47820
		MBD711	Os05g33550			AGO718	Os03g57560
		MBD712	Os04g11640		RNA-dependent RNA polymerase	RDR701	Os01g34350
		MBD713	Os04g11730			RDR702	Os01g10130
		MBD714	Os04g11510			RDR703	Os01g10140
		MBD715	Os08g37900			RDR704	Os02g50330
		MBD716	Os04g11530			RDR705	Os04g39160
		MBD717	Os04g52380		RNA polymerase IV (large)	NRPDA701	Os04g48370
		MBD718	Os08g10580			NRPDA702	Os09g38290

Histone variant	H2A.Z	HTA705	Os10g28230			NRPDA703	Os02g05880
		HTA712	Os03g06670		RNA polymerase IV (small)	NRPDB701	Os04g54840
		HTA713	Os03g53190			NRPDB702	Os08g07480
	H2A.X	HTA704	Os03g51200				
		HTA711	Os12g34510				
	H3.3	HTR701	Os06g06480				
		HTR709	Os02g25910				
		HTR710	Os02g25940				
		HTR711	Os03g27310				
		HTR712	Os06g04030				
		HTR714	Os12g22650				
		HTR715	Os12g22680				
	CENPA	HTR707	Os05g41080				
Histone methyltransferase (SET domain)	E(Z) [46]	SDG711	Os06g16390	Histone acetyltransferase	GNAT	HAG702	Os10g28040
		SDG718	Os03g19480			HAG703	Os04g40840
	ATXH + ATXR	SDG723	Os09g04890			HAG704	Os09g17850
		SDG721	Os01g11950		MYST	HAM701	Os07g43360
		SDG705	Os01g46700		CBP	HAC701	Os01g14370
		SDG739	Os03g07260			HAC703	Os02g04490
		SDG722	Os04g53700			HAC704	Os06g49130
		SDG701	Os08g08210		TAF_{II} 250	HAF701	Os06g43790
		SDG741	Os10g27060	Histone deacetylase [21]	RPD3/HDA1	HDA701	Os01g40400
		SDG720	Os01g73460			HDA702 [25]	Os06g38470
		SDG730	Os02g03030			HDA703 [25]	Os02g12350
		SDG717	Os12g41900			HDA704	Os07g06980

(continued)

Table 9.1 (continued)

	Class	ChromDB name	Tigr locus		Class	ChromDB name	Tigr locus
	ASHH+ASHR	SDG724	Os09g13740			HDA705	Os08g25570
		SDG708	Os04g34980			HDA706	Os06g37420
		SDG725	Os02g34850			HDA707	Os01g12310
		SDG716	Os03g49730			HDA709	Os11g09370
		SDG740	Os08g10470			HDA710 [25]	Os02g12380
		SDG736	Os02g39800			HDA711	Os04g33480
		SDG707	Os08g34370			HDA712	Os05g36920
	SUVH+SUVR [38]	SDG704	Os11g38900			HDA713	Os07g41090
		SDG713	Os03g20430			HDA714	Os12g08220
		SDG709	Os01g59620			HDA716	Os05g36930
		SDG728	Os05g41170		HD2	HDT701 [20]	Os05g51830
		SDG733	Os11g03700			HDT702	Os01g68104
		SDG734	Os12g03460		SIR2	SRT701 [28]	Os04g20270
		SDG726	Os07g25450			SRT702	Os12g07950
		SDG715	Os08g45130	Histone ubiquitination protein	Group A	HUPA701	Os04g46450
		SDG714 [42]	Os01g70220		Group B	HUPB701	Os05g08960
		SDG703	Os04g34990			HUPB702	Os03g57790
		SDG710	Os08g30910			HUPB703	Os07g07240
		SDG727	Os09g19830	Chromatin remodeling factor (SNF2 domain) [89]	Snf2	CHR707	Os02g02290
		SDG706	Os02g47900			CHR719	Os05g05230
		SDG729	Os01g56540			CHR720	Os06g14406
		SDG742	–		ISWI	CHR727	Os05g05780
		SDG712	Os02g40770			CHR728	Os01g27040
	Others	SDG738	Os04g34610		CHD	CHR702	Os06g08480
		SDG731	Os07g28840			CHR703	Os01g65850
		SDG732	Os09g38440			CHR705	Os07g46590
Histone arginin methyltransferase [88]	OsPRMT1	PRMT703	Os09g19560			CHR729 [50a]	Os07g31450
	OsPRMT3	PRMT710	Os07g44640		DDM1	CHR741	Os03g51230
	OsPRMT4	PRMT702	Os07g47500			CHR746	Os09g27060
	OsPRMT5	PRMT708	Os02g04660		Others	CHR701	Os02g06592

	OsPRMT6a		Os10g34740	CHR704	Os01g01312
	OsPRMT6b		Os04g58060	CHR706	Os01g57110
	OsPRMT7	PRMT709	Os06g01640	CHR708	Os01g72310
	OsPRMT10		Os06g05090	CHR709	Os02g46450
Histone demethylase (Jumomji family)	KDM5/IARID	JMJ703 [60a]	Os05g10770	CHR710	Os02g32570
		JMJ704	Os05g23670	CHR711	Os03g01200
		JMJ708	Os06g51490	CHR712	Os04g59620
	KDM4/JMJD2	JMJ706 [49]	Os10g42690	CHR713	Os05g15890
		JMJ707	Os02g46930	CHR714	Os04g47830
		JMJ701	Os03g05680	CHR715	Os04g53720
		JMJ702	Os12g18150	CHR717	Os10g31970
		JMJ705	Os01g67970	CHR721	Os07g44210
	KDM3/JMJD1	JMJ715	Os03g31594	CHR722	Os07g49210
		JMJ716	Os03g22540		
		JMJ718	Os09g22540	CHR724	Os07g44800
		JMJ719	Os02g01940	CHR725	Os08g08220
		JMJ720	Os02g58210	CHR726	Os07g40730
	JmjC domain only	JMJ709	Os01g36630	CHR730	Os03g06920
		JMJ711	Os03g27250	CHR731	Os07g32730
		JMJ710	Os11g36450	CHR732	Os03g22900
		JMJ717	Os08g39810	CHR733	Os02g52510
		JMJ713	Os01g56640	CHR735	Os04g09800
		JMJ714	Os09g31050	CHR736	Os07g25390
		JMJ712	Os09g31380	CHR737	Os06g14440
Histone demethylase (LSD1)		HDMA701	Os04g47270	CHR739	Os07g48270
		HDMA702	Os02g51880	CHR740	Os02g43460
		HDMA703	Os08g04780	CHR742	Os05g32610
		HDMA704	Os10g38850	CHR743	Os08g14610
				CHR745	Os01g44990

acetylation leads to chromatin compaction and gene repression [17] (Fig. 9.2). The dynamic modulation of histone acetylation in plants has been shown to be important for plant gene expression in responding to environmental conditions including light, temperature, biotic, and abiotic stresses [18, 19]. In rice, acetylation of H3 lysine 9 (H3K9) and H4 lysine 12 (H4K12) is elevated in genes located in euchromatic regions [20], suggesting that these markers are associated with active genes. Dynamic and reversible changes in histone H3 acetylation occur at submergence-inducible genes in rice [21]. Recent results have revealed a function of histone acetylation in circadian regulation of rice gene expression [22].

Histone acetylation homeostasis is regulated by antagonistic actions of histone acetyltransferases (HAT) and histone deacetylases (HDAC) (Fig. 9.2). Although HAT function in rice has not been reported, several rice HDAC genes have been studied [23]. The rice genome contains at least 19 HDAC genes belonging to three classes [24]. Among them, two have primary homology to yeast HDAC groups: RPD3 (Reduce Potassium Dependency 3), and SIR2 (Silent mating-type Information Regulation 2). The third group known as the HD2 class is only found in plants [25]. Expression and functional studies suggest that individual rice HDAC genes have specific development functions that may be divergent from the *Arabidopsis* homologues. Expression of rice HDAC genes shows tissue/organ-specificity. Most of the HDAC genes are responsive to drought or salt stresses and some of them display diurnal expression. Over-expression of *OsHDAC1* (also called *HDA702*), a RPD3 class member, leads to increased growth rate and altered architecture in transgenic rice [26, 27]. *OsHDAC1* deacetylates histone H3 lysine 9 (H3K9), lysine 14 (H3K14) and lysine 18 (H3K18) and histone H4 lysine 5 (H4K5), lysine 12 (H4K12) and lysine 16 (H4K16). However, over-expression of several other rice RPD3 members does not produce any visible phenotypes. In contrast, down-regulation of a few RPD3 members causes different developmental defects [23].

SIR2 proteins are NAD+-dependent HDACs, some of which have been found to be involved in metabolic regulation and in increasing lifespan in yeast and animals [28]. In rice or *Arabidopsis* only two *SIR2* genes have been identified [24]. Because the expression pattern of the two rice genes (*SRT701* and *SRT702*) and the subcellular localization of the proteins are different [23, 29, 30], the two genes are likely to have distinct functions. Down-regulation of *SRT701* by RNAi induces H3K9 acetylation, but reduces H3K9 dimethylation (see below for histone methylation) on many loci including transposable elements [30]. Transcription of many transposable elements and some of the hypersensitive response-related genes is activated in the RNAi plants, indicating that in wild-type rice plants transposons and cell death-related genes might be amongst the primary targets of SRT701, suggesting that SRT701-mediated histone deacetylation is an important component for transposon repression in rice.

Rice *HDT701* (*OsHDT1*) belongs to the plant-specific HD2 class of HDACs. Its expression displays a circadian rhythm [22]. Over-expression or down-regulation of the gene does not affect plant growth in an elite hybrid rice parent but the over-expression leads to early flowering of the hybrid under long day conditions [22]. Increased OsHDT1 levels repress the long day flowering repressors *OsGI* and *Hd1* whose expression is increased in the hybrid (a so-called "nonadditive" effect), likely due to increased acetylation levels over the genes. *OsHDT1* over-expression promotes histone H4 deacetylation over *OsGI* and *Hd1* during their peak expression phases in the hybrid and has an effect on nonadditive expression of many other genes in the hybrid [22]. It is possible that OsHDT1 is involved in epigenetic control of parental genome interaction for differential gene expression.

2.2 Histone Methylation and Histone Lysine Methyltransferases in Rice

Histone lysine methylation is an important epigenetic modification with both activating and repressive roles in gene expression [31].

Histone lysine residues can be mono- di- or trimethylated. For instance, H3K9 can be found at mono (H3K9me1)-, di (H3K9me2)-, or trimethylated (H3K9me3) state. Each methyl state may have a different function for genome activity. In plants, H3K9me2 is almost exclusively associated with heterochromatin regions (Fig. 9.2), while H3K9me3 is associated with genes. Trimethylated H3 lysine 27 (H3K27me3) is negatively correlated with gene expression, whereas trimethylated H3 lysine 4 (H3K4me3) and lysine 36 (H3K36me3) are associated with active genes [32] (Figs. 9.1 and 9.2).

2.2.1 H3K4 Methylation

In *Arabidopsis*, H3K4 methylation is found over about two-thirds of genes and is underrepresented in repeats and transposon-rich regions of the genome [33]. While H3K4 monomethylation (H3K4me1) and dimethylation (H3K4me2) are associated with both active and inactive genes, H3K4me3 is mostly correlated with active genes. H3K4me3 and H3K4me2 are detected mostly at the promoter and the 5′ end of genes (Fig. 9.1). In rice about half of the protein-coding genes have di- and/or trimethylated H3K4 based on the analysis of two chromosomes [34]. Rice genes with predominant H3K4me3 methylation are actively transcribed, whereas genes with predominant H3K4me2 methylation are transcribed at moderate levels [9, 34]. It has been shown that H3K4me3 increases over inducible genes in plants upon application of inductive signals [21].

Enzymes involved in histone methylation usually contain a motif called a SET domain, which is named after 3 *Drosophila* genes: Su(var)3-9, Enhancer of zeste (E(Z)), and Trithorax, the mutation of which either enhance or suppress epigenetic mutations [35] (Fig. 9.2). A large number of SET-domain genes are identified in rice and *Arabidopsis* genomes (Table 9.1). Trithorax proteins are a group of methyltransferases for H3K4 methylation. *Arabidopsis* TRITHORAX-RELATED1, 2 (ATX1, 2) and ATX-Related7 (ATXR7) have been shown to be involved in H3K4 methylation [36–38]. Other SET-domain proteins (SDG2) have been recently shown to be also involved in H3K4 methylation [39]. Rice homologues of ATX have been identified [40] (Table 9.1), while their function remains to be characterized.

2.2.2 H3K9 Methylation

Methylation of H3K9 is important for chromatin structure and gene regulation. H3K9me2 is found to be enriched in heterochromatic repetitive sequence regions, while H3K9me3 is distributed in the 5′ end of genes in euchromatic regions and is considered as a "mild" activating mark of gene transcription [41, 42] (Figs. 9.1 and 9.2). Drosophila Su(var)3-9 protein was the first identified histone lysine methyltransferase specific for H3K9 [43]. Plant genomes encode many *SUVH* genes [44]. *Arabidopsis SUVH*, also known as *KYP* (*KRYPTONITE*), and *SUVH5* and *SUVH6* encode activities of H3K9 mono- and dimethyltransferases [45–47]. The rice genome encodes 12 *SUVH* genes, among which *SDG714* is found to be involved in H3K9me2 and DNA methylation of *Tos17*, a *copia*-like retrotransposon [48]. A systematic study of rice *SUVH* genes indicated that different members display distinct function in histone H3K9 methylation, DNA methylation, and transposon silencing [44].

2.2.3 H3K27 Methylation

All three methylated states of H3 lysine 27 are generally associated with repressive chromatin. In *Arabidopsis*, H3K27me3 is associated with about 10 % of annotated genes that are expressed at low levels or repressed in a tissue-specific manner [42, 49], whereas H3K27me1 is mostly associated with silent transposable elements and repetitive sequences [50]. H3K27me3 is distributed all over the gene body region (Fig. 9.1). About a similar percentage of genes are marked by H3K27me3 in rice [9], which are mostly repressed genes [50a].

E(Z) homologues which are components of polycomb group (PcG) complexes are responsible for trimethylation of H3K27 (Fig. 9.2). Several homologues of E(Z) in *Arabidopsis* have been shown to behave as essential regulators of plant

developmental transitions by maintaining repression of key developmental regulatory genes [51]. Homologues of E(Z) and other PcG genes have been identified in rice [52]. Loss-of-function of rice E(Z) genes does not lead to similar defects found in mutants of *Arabidopsis* E(Z) homologues [52], suggesting that developmental function of these genes may not be conserved between different plant species. In *Arabidopsis*, two other ATX-Related genes, ATXR5 and ATXR6, are shown to be responsible for H3K27 monomethylation over repetitive sequences [50] (Fig. 9.2). The rice genome contains a higher proportion of repetitive sequences. Whether homologues of ATXR5 and ATXR6 or additional proteins are involved in H3K27me1 remains to be determined.

2.3 Histone Demethylases

Repression of active genes implies removal of methyl groups from H3K4me3, while activation of repressed genes may require demethylation of H3K27me3 (Fig. 9.2). Histone methylation is reversed by histone demethylases. LSD1 (Lysine specific demethylase 1) was the first histone demethylase to be identified to demethylate H3K4me1 and H3K4me2, in addition to H3K9me1 and H3K9me2 [53]. Four *LSD1* homologues are found in rice and *Arabidopsis*. *Arabidopsis LSD1* genes have been shown to be involved in flowering time regulation [54]. The function of rice LSD1 has not yet been determined. In addition, Jumonji C (jmjC) domain-containing proteins are found to function also as histone demethylases by removing di- and trimethyl groups [55, 56]. More than 20 jmjC protein genes are identified in rice and *Arabidopsis* [57, 58]. Two *Arabidopsis* jmjC proteins, JMJ14 and JMJ15 are shown to possess specific demethylase activity to reverse di- and trimethylated H3K4 [32, 59, 60]. Closely related homologues in rice have also been shown to demethylate H3K4 [60a].

Rice *JMJ706* (Os10g42690) and *Arabidopsis Increase in Bonsai Methylation1* (*IBM1*/*JMJ25*, At3g07610) have activities to remove methyl groups of di- and trimethylated H3K9 in vitro and/or in vivo [58, 61]. Mutations in the two genes produce severe developmental defects, suggesting that histone H3K9 demethylation is essential for normal plant development. Mutations in rice *JMJ706* affect floral organ number and seed development and lead to an increase of H3K9me2/3 [58]. In addition, the phenotype of rice *jmj706* mutants can be partially suppressed by RNAi of a few rice *SUVH* genes [44], indicating that SUVH proteins may form antagonistic couplets with JMJ706 to regulate the homeostasis of H3K9 methylation. It was recently shown that the *Arabidopsis REF6* gene that is closely related to rice *JMJ705* and *JMJ706* is involved in demethylation of H3K27me3 [62]. It remains to be determined whether these rice proteins also have a demethylase activity of H3K27me3.

3 Recognition of Histone Modifications

Histone modification modules are recognized by chromatin proteins that have activities to remodel chromatin structure to regulate gene transcription or induce heterochromatin formation (Fig. 9.2). Acetylated histone lysine residues (e.g., H3K14ac) are bound by bromodomain-containing proteins, such as histone acetyltransferase GCN5 [63]. The different methylated histone lysine residues are recognized by different chromatin protein modules including chromodomains and PHD (Plant HomeoDomain) fingers. For instance, in animal cells the chromodomain of Heterchromatin Protein1 (HP1) binds to H3K9me2 [64], while the chromodomain of the polycomb protein is associated with H3K27me3 [65]. However, in *Arabidopsis*, the chromodomain of LHP1 (LIKE HETEROCHROMATIN PROTEIN 1) interacts with H3K27me3 [42]. A subset of PHD finger-containing proteins are able to interact with H3K4me3 [66]. In addition, chromodomains can also recognize methylated H3K4. For instance, mammalian chromodomain protein CHD1 binds

to H3K4me3 to regulate gene activation [67]. A rice chromodomain protein CHD3 has been shown to be able to interact with both H3K4me2 and H3K27me3 [50a].

4 Interplay Between Chromatin Modifications

Regulation of chromatin remodeling processes involves functional interactions between multiple chromatin modifications (Fig. 9.2). For instance, H3K4me3 is positively associated with H3K9ac. H3K4me3 and H3K9ac are active marks, and represent a chromatin signature of active genes in plants [68]. In addition, H3K9ac is shown to be antagonistic to H3K9me2 in rice. Down-regulation of the HDAC gene *SRT701* not only increases H3K9ac but also reduces H3K9me2 leading to transcriptional activation of many transposable element-related genes in rice [30]. It is likely that deacetylation promotes H3K9 methylation required for transposable element silencing in rice. Genome-wide analysis indicates that H3K4me3 and H3K27me3 are mutually repulsive and antagonistic marks in plants [33, 68]. In rice, relatively few genes are co-modified by both marks [50a]. Interplay between the two histone modification marks is likely to play an important role in gene expression in rice [9]. This may imply physical or functional interactions between histone demethylases and other histone modification complexes as revealed in animal cells [31]. It remains to be known whether similar protein complexes exist in plant cells to coordinate methylation/demethylation of H3K4 and H3K27.

DNA methylation plays critical roles in epigenetic processes and is associated with histone methylation. DNA methylation requires unmethylated H3K4 nucleosomes in mammalian cells [69], while H3K27me3 seems to be antagonistic to DNA methylation in plants [70]. De novo DNA methylation can be triggered not only by small RNA but also by H3K9me2. In *Arabidopsis*, dimethylation of H3K9 by KYP is required for maintenance of CHG methylation by CMT3 [71]. Conversely, the H3K9 demethylase *IBM1*/*JMJ25* has a function to protect active genes from H3K9me2 and CHG methylation [72]. However, mutations of the rice homologue *JMJ706* that affect overall H3K9me2 did not affect DNA methylation in gene bodies (unpublished data), suggesting that JMJ706 may be functionally distinct from IBM1. By contrast, loss-of-function of a H3K4 demethylase gene *JMJ14* leads to loss of non-CG methylation at loci targeted by RNAi-directed DNA Methylation (RdDM) in *Arabidopsis* [73]. Therefore histone demethylation on different lysine residues may have distinct roles in DNA methylation regulation [57]. It remains to be known whether similar interactions are conserved in rice that has a larger amount of repetitive sequences and transposable elements than *Arabidopsis*.

5 Epigeneomic Variations, Inheritance and Epialleles

Closely related species may develop differences in their epigenetic systems during adaptation to different environmental niches. Studies of natural variation of DNA methylation in a number of other plant species have suggested that epigenetic variation among individuals with similar genotype can lead to phenotypic variation in response to varying environmental conditions [74]. Epigenetic adaptive responses to environmental cues can be transmitted to future generations [75, 76].

It has been shown that DNA methylation in genes is extremely polymorphic among 96 natural accessions of *Arabidopsis* [77]. More recent studies by examining spontaneously occurred variation in DNA methylation in *Arabidopsis* plants propagated by single seed descent for 30 generations have revealed that transgenerational changes in cytosine methylation occur at a high frequency [78, 79]. Transgenerational variation in DNA methylation that adversely affects gene expression may generate new epigenetic alleles (epialleles) leading to phenotypic variation without DNA sequence change. DNA methylation-induced silencing of protein-coding genes gives rise to epialleles that can be inherited through

meiosis [80]. Two examples of meiotically heritable epialleles resulting in morphological variation are the *peloric* (in *Linaria vulgaris*) and *colorless non-ripening* (in *Solanum lycopersicum*) loci which are spontaneous epigenetic silencing events [81, 82]. In rice, the epiallele of *DWARF1* (*D1*), *Epi-d1*, causes a metastable dwarf phenotype [83]. The silenced state is correlated with repressive histone and DNA methylation marks in the *D1* promoter region. It has been recently shown that the expression level of *OsSPL14* (*SQUAMOSA PROMOTER BINDING PROTEIN-LIKE14*) is important to regulate panicle branching and grain yield in rice [83]. Differences in DNA methylation and histone modifications on the *OsSPL14* locus are likely to be responsible for the expression difference of the gene between two *japonica* rice varieties that differ in grain numbers per panicle. This case demonstrates that epigenetic mutations may be an important source for variation of important agronomic traits in rice.

6 Perspectives

In the long history of rice evolution, domestication and selection, epigenomic variations may have been generated and may have contributed to phenotypic differences and variations in complex traits among different species, subspecies, and cultivars. In addition, epigenomic variation among individuals with similar genotypes can drive phenotypic variation in response to varying physical, biotic, and abiotic environments. Many morphological and adaptive phenotypes may be dependent on different epialleles. Therefore, investigating rice and other *Oryza* epigenomes will be important to identify specific epigenetic marks and epialleles involved in important agronomic and adaptive traits. Functional characterization of rice chromatin modification regulators (writers, erasers, and readers) has just started and remains to be an important research field to understand the mechanism of establishment, maintenance, recognition and inheritance, or erasure of rice epigenomes. In fact, understanding how established epigenomic marks corresponding to specialized plant cell types or responding to induction by specific environmental cues can be memorized during subsequent cell divisions and inherited to next generations represents an essential research aspect of epigenetics in the future.

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