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REVIEW ARTICLE

The impact of histone post-translational modifications on developmental gene regulation

Mark N. Cruickshank · Paul Besant · Daniela Ulgiati

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Abstract Eukaryotic genomic DNA is orderly compacted to fit into the nucleus and to inhibit accessibility of specific sequences. DNA is manipulated in many different ways by bound RNA and proteins within the composite material known as chromatin. All of the biological processes that require access to genomic DNA (such as replication, recombination and transcription) therefore are dependent on the precise characteristics of chromatin in eukaryotes. This distinction underlies a fundamental property of eukaryotic versus prokaryotic gene regulation such that chromatin structure must be regulated to precisely repress or relieve repression of particular regions of the genome in an appropriate spatio-temporal manner. As well as playing a key role in structuring genomic DNA, histones are subject to site-specific modifications that can influence the organization of chromatin structure. This review examines the molecular processes regulating site-specific histone acetylation, methylation and phosphorylation with an emphasis on how these processes underpin differentiation-regulated transcription.

Keywords Cell differentiation · Histones · Chromatin · Transcription factors

Introduction

Histones were originally identified as the major basic protein fraction of chromatin that forms a salt-like combination with nucleic acid (Kossel 1910). The basic unit of chromatin, the nucleosome, consists of approximately 146 bp of DNA wrapped 1.75 turns around an octameric complex of core histones H2A, H2B, H3 and H4 family members. The prototype nucleosome consists of a $(H3)_2(H4)_2$ tetramer with two (H2A)(H2B) heterodimers binding either side (Kornberg 1974). Nucleosomes are separated by linker DNA forming a bead-like structure with a diameter of $\sim$11 nm (Oudet et al. 1975; Turner 2005). Each core histone contains a fold domain comprising a globular structured three-helix region which contacts DNA, while unstructured N-terminal tails protrude from the core particle contacting neighboring nucleosomes (Luger et al. 1997a, b; Richmond and Davey 2003). Histones are dynamically regulated to promote or repress processes such as transcription (Heintzman et al. 2009; Yang et al. 2009), replication (Corpet and Almouzni 2009), repair (Ahel et al. 2009; van Attikum and Gasser 2009) and recombination (Wang et al. 2009b). The nature of histone-directed gene regulation is extremely variable. Histones are subject to diverse post-translational modifications (PTMs) (Cosgrove and Wolberger 2005) and can also be replaced within a nucleosome by variant sub-types (Bell and Schubeler 2009; Goldman et al. 2010).

Chromatin compaction is dynamically regulated through the cell cycle and in response to either extrinsic or developmental stimuli. During mitosis, chromatin condenses such that chromosomes become discreetly visible structures under a light microscope. As cells make the transition to interphase, chromosomes disperse unevenly so that some regions of the genome remain condensed while other regions become diffuse. Heterochromatin is the condensed fraction containing repressed regions of the genome in contrast to euchromatin which is diffuse and metabolically active. Constitutively assembled heterochromatin includes

M. N. Cruickshank · P. Besant · D. Ulgiati (✉)
Biochemistry and Molecular Biology, School of Biomedical, Biomolecular and Chemical Sciences,
University of Western Australia, 35 Stirling Highway,
Crawley, WA 6009, Australia
e-mail: daniela.ulgiati@uwa.edu.au

genomic DNA located at or near the telomeres and pericentric regions of the chromosomes as well as other regions rich in repetitive sequences (Deng et al. 2009; Loyola et al. 2009). Repression of these regions is critical to prevent spurious recombination of genomic DNA which partly explains why heterochromatin formation promotes stability of the genome. In contrast, facultative heterochromatin, or silenced euchromatin, refers to genomic regions that are condensed only in a subset of cells. The passage of genomic DNA from facultative heterochromatic to euchromatic regions and vice versa plays a key role in modulating transcriptional responsiveness to external stimuli. Moreover, alterations to the packaging of genomic DNA within chromatin can be replicated during cell division so developmental programming of chromatin can be stably inherited (Craig 2005; Grewal and Jia 2007).

In eukaryotes, processes that require access to genomic DNA are regulated by chromatin structure. Histone PTMs are important regulators of chromatin structure and are implicated in regulating transcription (Edmunds et al. 2008; Snykers et al. 2009), pre-mRNA splice site selection and polyadenylation site selection (Spies et al. 2009), DNA replication timing during mitosis (Goren et al. 2008; Lande-Diner et al. 2009), DNA repair (Ikura et al. 2000; Stucki and Jackson 2004), recombination during meiosis (Borde et al. 2009; Buard et al. 2009) and somatic recombination in lymphocytes (Giambra et al. 2008). Indeed, euchromatin and heterochromatin are both highly heterogenous with respect to nucleosome phasing and histone PTMs (reviewed in Campos and Reinberg 2009). This review examines the molecular processes regulating site-specific acetylation, methylation and phosphorylation of the core histones, with an emphasis on how these processes underpin differentiation-regulated transcription.

Regulation of chromatin structure by nucleosomes

At the simplest level, nucleosomes regulate genomic DNA accessibility by forming a barrier so the positioning and strength of DNA-nucleosome interactions can affect access to regulatory sequences. Mechanisms directly targeting the primary nucleosome organization involve two types of enzymes which either covalently modify histones (for example acetylases or methylases) or utilize energy from ATP hydrolysis to disrupt DNA–nucleosome interactions (SWI/SNF-type enzymatic complexes) (Horn and Peterson 2002). Histone-modifying and SWI/SNF remodeling enzymes are targeted to specific sites in the genome in complexes with DNA-binding transcription factors (TFs) (Khorasanizadeh 2004). Additionally, it is now becoming apparent that site-specific recruitment of these histone-modifying complexes can also be targeted by non-coding RNA molecules (Hawkins et al. 2009; Mattick et al. 2009).

Upper hierarchies of regulation include nucleosome array folding (Bassett et al. 2009; Tremethick 2007) and compartmentalization of functional domains in the nucleus (Fromm et al. 2009; Mutskov and Felsenfeld 2009). The first level of packaging DNA by nucleosomes has been resolved at the atomic level, however, the arrangement of higher order chromatin fibers is poorly understood. The second level of compaction, which involves a string of nucleosomes folding into a fiber with an approximate diameter of 30 nm, is regulated by linker histones (e.g. H1, H5) that bind to DNA between adjacent nucleosomes to promote condensation (Grigoryev et al. 2009; Kruithof et al. 2009; Routh et al. 2008). On the other hand, several high mobility group (HMG) proteins are known to counteract chromatin condensation by linker histones (Belova et al. 2008; Rochman et al. 2009). Like the core histones, linker histones and HMG proteins are also subject to diverse PTMs and these can regulate chromatin structure, however, these will not be discussed in this review (see references Wood et al. 2009; Zhang and Wang 2008).

Looped 30 nm fibers may be further condensed into heterochromatic fibers that are 100–400 nm thick. This type of hierarchical folding might allow for partitioning of chromosomal domains into regions that are regulated with varying degrees of autonomy. Various classes of DNA regulatory sequences including insulators (Ishihara et al. 2006), matrix attachment regions (MARs) (Cai et al. 2003) and locus control regions (LCRs) (Ho et al. 2006) have been proposed to regulate looped domain structure to control long-range regulatory processes. Regulated compaction of genomic DNA at each level may provide different levels of control of chromatin accessibility and gene activity (Peterson and Laniel 2004).

While nucleosomes are simple repeating units of chromatin, they are not identical. Variant copies of H2A and H3 have been identified and these are proposed to play a role in regulating chromatin structure (Altaf et al. 2009; Loyola and Almouzni 2007; Zilberman et al. 2008). Furthermore, a wide range of covalent modifications have been identified on each of the histones in vivo. For example, lysine residues may have acetyl, methyl, SUMO or ubiquitin moieties covalently attached. Similarly serine and threonine residues may be phosphorylated, arginine residues may be methylated and glutamate residues may be ADP-ribosylated. From these observations a theory known as the histone code has been proposed to explain regulation of chromatin structure (Jenuwein and Allis 2001; Strahl and Allis 2000) which predicts that:

- Distinct modifications to histones induce altered interaction affinities with chromatin associated proteins or DNA.
- Many different sites are available for modification on the same histone molecule. Modifications may be interdependent and generate various combinations within a given nucleosome.
- Chromatin structural variation associated with euchromatin or heterochromatin are dependent upon the local concentration and combination of differentially modified nucleosomes which regulates recruitment of chromatin modifying factors.

Advances in techniques such as chromatin immunoprecipitation and immuno-fluorescence, which explore site specific histone and non-histone PTMs within chromatin, now suggest that the histone code constitutes part of a wider PTM protein code (Margueron et al. 2005). This hypothesis has gained traction from observations that non-histonal chromatin associated proteins are similarly subject to an array of PTMs. Moreover, PTMs of TFs and co-factors are targeted during differentiation in an analogous manner to histones and can regulate protein–protein and protein–DNA interactions (Pradhan et al. 2009; Sampath et al. 2007; Wang et al. 2009a).

Regulation of RNA polymerase II-dependent transcription

Restricted stage- and lineage-dependent transcription involves the concerted action of multiple TF binding sites and the cell specific background of signalling molecules, TFs and co-regulators. Specificity of developmental transcriptional activity also depends upon chromatin structural variation at *cis*-elements as this variation can modulate TF assembly on target sequences. Gene-specific factors may either recruit components of the pre-initiation complex (PIC) which includes TATA-binding protein (TBP), TBP-associated factors (TAFs) and RNA polymerase II (RNA polII), or modify the local chromatin structure to indirectly regulate PIC factor binding (Chen and Hahn 2004; Hahn 2004).

Components of the PIC are assembled in distinct steps, however, the nature of PIC assembly is gene specific (de la Serna et al. 2005; Hatzis and Talianidis 2002). Furthermore, assembly of components of the PIC at developmentally restricted or inducible promoters, correlates with the appearance of specific histone PTMs at regulatory elements (Flajollet et al. 2006; Martens et al. 2003; Soutoglou and Talianidis 2002). Therefore, transcription initiation is regulated by a sequence of events orchestrated by core promoter sequences and gene specific factors leading to histone PTMs and nucleation of the PIC (Lefevre et al. 2005; Woychik and Hampsey 2002).

Formation of the PIC at core promoter sequences is insufficient to effect transcript elongation, as several mechanisms restrict gene activation at this stage (Wittmann et al. 2005). For instance, nucleosomes impede the release of RNA polII from the proximal promoter in vivo and also slow the rate of transcription in vitro (Kireeva et al. 2005). A class of elongation factors function to alleviate this barrier and regulate transcriptional activity by stimulating the release of RNA polII from the proximal promoter (Gerber and Shilatifard 2003). These processes can involve extensive disruption to the nucleosome core particle (Saunders et al. 2003), histone PTMs (Pavri et al. 2006) and SWI/SNF-dependent remodelling (Martens et al. 2003). Therefore, recruitment of histone modifying enzymes and SWI/SNF-complexes to nucleosomes near transcriptional start sites may regulate several aspects of transcription initiation and elongation by RNA polII.

Differentiation regulated histone post-translational modifications

Experimental evidence linking histone PTMs and transcription has been accumulating since the 1960s (Allfrey et al. 1963; Frenster et al. 1963; Goldberg and Atchley 1966; Littau et al. 1964), however, the molecular mechanisms underlying differentiation-induced histone regulation are only beginning to emerge. Recent studies have demonstrated that gene-specific histone modifications are established in response to developmental cues to coordinate the expression of co-regulated genes. In fact, differentiation-induced gene expression involves sequential alterations to histones occupying regulatory elements which first prime and/or poise the locus for activation and then subsequently activate and in some cases attenuate transcriptional activity. There are a variety of PTMs to histones (reviewed in references Cosgrove et al. 2004; Mellor et al. 2008; Suganuma and Workman 2008; Weake and Workman 2008) and variant sub-types of H3 and H2A (reviewed in references Hake and Allis 2006; Loyola and Almouzni 2007) that may regulate transcription and other processes that require access to DNA within chromatin. Furthermore, a variety of histone PTMs have been detected that have not been functionally characterized. In fact, molecular processes linking specific histone PTMs with DNA-dependent biological processes have been intensively studied, however, clear cause-consequence relationships have largely remained elusive. Indeed, the presence of specific histone PTMs do not necessarily point to a biological function in isolation. On the other hand, biologically relevant histone PTMs have been inferred when they

are specifically enriched at important regulatory regions or broader chromosomal domains that may, for instance, demarcate euchromatin and heterochromatin. The following discussion focuses on well characterized processes influencing differentiation-regulated transcription including histone acetylation and methylation. In addition, we will introduce the notion that histone phosphorylation appears to play a role in cell cycle control which may be important in regulating cellular differentiation, however, these processes are poorly understood at present.

Histone acetylation

Histone acetylation contributes to the establishment or maintenance of a permissive environment for transcription, whilst not necessarily causing transcriptional activation (Grunstein 1997). In contrast, histone deacetylation promotes chromatin condensation and repression (Tse et al. 1998). Acetylation of histones can stimulate transcriptional activity directly by relaxing DNA-nucleosome interactions which form a barrier to factor recruitment and transcription elongation (Legube and Trouche 2003). In addition to the electro-static influence of lysine-acetylation, histones bearing this modification are specifically recognized by many proteins containing a bromodomain (Bottomley 2004; Taverna et al. 2007). This 110 amino acid protein domain binds specifically to acetylated lysine residues including those on histones. Bromodomains are present in many transcription co-activators including HATs (Bottomley 2004; Dhalluin et al. 1999), BET nuclear factors (BRD2, BRD4, BDF1) (Dey et al. 2003; Maruyama et al. 2002) and SWI/SNF remodeling factors (BRM, BRG1) (Grune et al. 2003; Kadam and Emerson 2003). The bromodomain stabilizes the interaction of these factors with acetylated histones to maintain accessible chromatin structures (Hassan et al. 2001; Winston and Allis 1999). Recently, Brdt, a testis-specific Bet family member, was shown to selectively recognise H4 histone tails bearing two or more acetyl-lysine with highest affinity detected for H4 acetylated at K5/K8 (Moriniere et al. 2009). This interaction was mediated by a single bromodomain implying that a single module can interact with histones differentially depending on the level of acetylation. Therefore, acetylation of core histones can enhance transcription activity by directly enhancing accessibility of bound DNA and also indirectly by stabilizing interaction with co-factors.

Genomic patterns of histone acetylation are maintained by opposing classes of enzymes, the histone acetylatransferases (HATs) and histone deacetylases (HDACs). These are distributed in a targeted manner throughout chromatin such that at any given genomic region the level of histone acetylation results from a balance of their opposing activities. Interestingly, genome-wide analyses of HDAC and HAT distribution in human T-cells has shown that binding of both HDACs and HATs to promoter regions correlated with transcriptional activity (Wang et al. 2009c). The authors suggest that cycles of transcription initiation involve acetylation of histones followed by deacetylation to "reset" chromatin after release of RNA polII. Furthermore, HDAC6 activity across the transcribed region of active genes was detected paralleling RNA polII activity. The authors speculate that this may prevent cryptic transcription start site selection (Wang et al. 2009c). HAT and HDAC enzymes can be purified from large multi-subunit complexes which contain components that provide specificity (both genomic location and substrate target) and enzymatic cofactors. In addition to targeting lysine residues within histones (Fig. 1), these complexes can also acetylate other proteins including many TFs (Legube and Trouche 2003; Wang et al. 2005).

The HATs are divided into five main groups—Gcn5-related acetylatransferases (GNATs), p300/CREB-binding protein (CBP) HATs, general TF HATs, nuclear hormone-related HATs and MYST-related HATs (Sterner and Berger 2000). Human GNATs include GCN5 and PCAF which function as co-activators for a subset of genes. On the other hand, p300 and CBP are ubiquitously expressed global HATs that are recruited promiscuously to activate transcription (Legube and Trouche 2003). General TFs are required for the assembly of RNA polII initiation complexes and many of these components have been found to possess intrinsic HAT activity. The p160 family of nuclear receptor co-activators, including SRC-1, ACTR and TIF2,

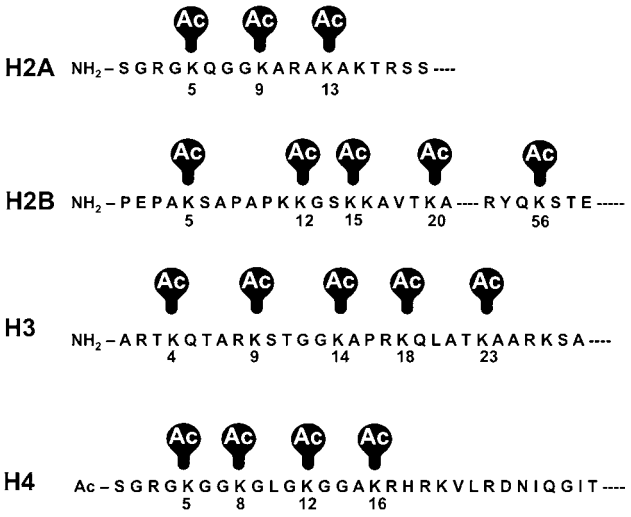

Fig. 1 Histone H2A, H2B, H3 and H4 acetylation target sites. The amino acid sequence of H2A, H2B, H3 and H4 amino-terminal tails is shown with potential acetylation sites numbered below—lightbulb motifs representing lysine acetylation are shown above sequence

also utilize intrinsic HAT activity to regulate target genes upon ligand binding (Rosenfeld and Glass 2001).

Human HDACs belong to four classes based on homology to the yeast HDACs; Rpd3 (class I—HDAC1, HDAC2, HDAC3 and HDAC8), Hda1 (class II—HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10) and Sir2 (class III—human sirtuin proteins SIRT1-7) (Marmorstein and Roth 2001). HDAC11 shares homology with both yeast Rpd3 and Hda1 and is the sole class IV HDAC identified to date (Yang and Seto 2008). Class I and II HDACs generally function as co-repressors and these are sensitive to inhibition by chemicals like Trichostatin A (TSA). Class III HDACs are NAD^+-dependent enzymes that may play a crucial role in gene silencing and are insensitive to TSA-inhibition (Denu 2005; Kang et al. 2005). During differentiation, HDAC enzymes are regulated by a variety of mechanisms including expression level (Wada et al. 2009; Yoo et al. 2006), *S*-nitrosylation (Nott et al. 2008), nuclear translocation (Hisahara et al. 2008; Jensen et al. 2009) and their association with multiprotein complexes (Chang et al. 2008; Guo et al. 2009).

Differentiation regulated histone acetylation

It is well documented that histone H3 and H4 acetylation play a key role in regulating gene activation at developmentally restricted loci as well as broadly expressed genes (Clayton et al. 2006; Fish et al. 2005; Kanno et al. 2004). In developing or differentiated cells, H3 and H4 acetylation of nucleosomes near TSSs generally correlates with transcriptional activation of stage-restricted genes. In particular, H3 acetylation (H3K9/14) near the TSS of genes activated during differentiation correlates with stage-restricted transcription in many cell types such as neurons (Attia et al. 2007), skeletal muscle (Mal and Harter 2003), adipocytes (Nakade et al. 2007), pancreatic β cells (Chakrabarti et al. 2003), enterocytes (Hatzis and Talianidis 2002; Soutoglou and Talianidis 2002), B- (Green et al. 2006; Lim et al. 2006) and T-lymphocytes (Shi et al. 2008).

While acetylation of histones near the TSS appears to be a general mechanism to activate cell- and stage-specific transcription, this phenomenon is not definitively correlated with gene activation. Hyperacetylation of histones has been reported at several classes of regulatory elements, including promoter sequences, prior to developmental gene activation. In particular, highly inducible genes may be acetylated at promoter regions prior to transcription, while various classes of regulatory elements may also be acetylated. For example, Liang and co-workers report enhanced acetylation of histones directly downstream of the *IL-1β* TSS in resting monocytes prior to transcriptional activation. They also found a modest increase in H3 acetylation upstream of the *IL-1β* TSS upon stimulation of monocyte cells (Liang et al. 2006). On the other hand, differentiation of naïve T cells into either Th1 or Th2 effector cells results in complex patterns of expression and histone modifications across broad regions of the *IFN-γ* and *IL-4* loci (Ansel et al. 2003; Bream et al. 2004; Chang et al. 2008; Shi et al. 2008). During early differentiation, transcription of both cytokine genes occurs at low levels with widespread increases in histone H3 acetylation across both genomic regions. As differentiating Th cells adopt either the Th1 or Th2 phenotypes, both histone acetylation and transcription are selectively maintained at either the *IFN-γ* or the *IL-4* genes respectively (Avni et al. 2002). Subsequently, resting Th1 and Th2 cells downregulate transcription of each of these cytokine genes, however, the histone acetylation marks persist across either the *IFN-γ* and *IL-4* regulatory regions respectively. It has been suggested that histone acetylation of these genomic regions is a marker of Th cell differentiation and transcriptional competence rather than transcription *per se* (Fields et al. 2002). A recent report characterising genome-wide histone PTMs, including H3K9 acetylation and H3K4 methylation, in resting and stimulated T cells supports this notion (Lim et al. 2009b). In this study, inducible genes showed higher levels of active histone marks including histone H3K9 acetylation in cells prior to stimulation. RNA polII could be detected at these promoters and was further enriched following stimulation, while the histone PTMs examined generally maintained their active marks without obvious changes. It is possible that these observations reflect a common difference in the control of histone acetylation among stably expressed cell-specific genes compared to highly inducible genes.

Remarkably, a genome-wide study of HATs and HDACs in T-cells determined that neither HDAC nor HATs could be detected at a sub-set of silenced genes, while on the other hand, high levels of both HATs and HDACs were associated with actively transcribed genes (Wang et al. 2009c). Furthermore, low levels of HDAC and HAT activities were observed at a sub-set of non-expressed genes suggesting transient association of these co-factors. Treatment with HDAC inhibitors demonstrated that nearly all genes lacking HDAC and HAT activity were insensitive to acetylation, in contrast, many genes associated with low level HDAC and HAT activity were highly acetylated following treatment. This sub-set of genes that were susceptible to acetylation were not necessarily transcriptionally activated, but still showed enhanced recruitment of RNA polII specifically at promoter regions. These studies suggest that histone deacetylation at "primed" genes functions to prevent binding of the PIC (Wang et al. 2009c).

Specific regulatory regions located distally or proximally to core promoter sequences may also be packaged in

nucleosomes which are acetylated prior to developmental gene activation. For example, Talianidis and co-workers have shown that, in vitro, cells capable of spontaneous differentiation and activation of a key liver enriched TF, HNF-4α, are primed for expression by ordered nucleosome positioning at both the *HNF-4α* promoter and an upstream enhancer region (~6.5 kbp upstream of the *HNF-4α* TSS), as well as selective histone acetylation at the enhancer, but not the promoter (Hatzis and Talianidis 2002). During differentiation, histone H3 and H4 acetylation of the *HNF-4α* promoter correlates with the timing of transcriptional activation. In addition, transcription of the β-globin locus has been extensively studied as a model of developmental gene activation since expression of genes within this cluster are restricted to erythroid cells and show differential expression during embryonic development and adult erythropoiesis (Bender et al. 2000; Bulger et al. 2003). Histone acetylation at this locus is not confined to the β-lobin gene promoters, but rather extend over tens of kilobases termed "hyperacetylated domains". Despite extensive studies examining developmental β-globin gene expression, the mechanisms regulating these domains is not completely characterised. These regions, however, are thought to be hyperacetylated during erythroid development prior to gene activation within this cluster (Fromm et al. 2009).

Additionally, MAR elements have been identified which play a role in regulating histone acetylation and transcription induction over vast genomic regions. For example, a specific MAR element and the MAR-binding protein, SATB1, are necessary both for lineage-specific silencing of a subset of genes (including *myc*), and also for thymocyte-specific gene transcription and long-range histone acetylation (Cai et al. 2003). Since SATB1 interacts with either a HAT (PCAF) or a HDAC (HDAC1) in a phosphorylation-dependent manner, global gene regulation by SATB1 may be modulated by the protein kinase C pathway and by targeting either HATs or HDACs to SATB1 sites (Pavan Kumar et al. 2006). Another class of regulatory element, referred to as insulators or barriers, are also important in regulating histone acetylation over long distances. These elements bind CTCF, demarcate physically linked genes to enable independent gene regulation and block the effect of neighbouring regulatory elements such as enhancers (Hou et al. 2008; Zhao et al. 2006). Insulators are depleted in nucleosomes and are also associated with foci of high levels of histone acetylation which may be constitutive and not necessarily correlated with cell type or gene expression (Litt et al. 2001b). Furthermore, CTCF has been identified as a key regulator of genomic imprinting at the *H19/Igf2* locus such that CTCF binding is necessary for establishing maternal-specific allelic transcription and histone modifications, including H3K9 acetylation, across several distant regulatory regions (Han et al. 2008).

Thus, several classes of regulatory elements are subject to histone acetylation during differentiation, however, the functional outcome is variable. Histone acetylation may correlate either with transcription activation or differentiation stages prior to gene activation and also may have either long-range or localised effects on genomic acetylation patterns. Acetylation of histones may therefore, provide a means whereby TF complexes (including regulatory RNAs) combine sequentially to execute activation of gene expression at specific stages of differentiation, or alternatively, enable rapid induction of gene expression which is necessary in specific cell lineages.

Histone methylation

Covalent methylation of histones occurs on arginine and lysine residues. Unlike lysine acetylation, these modifications may not significantly influence chromatin structure directly since the methyl group is relatively small and does not neutralize arginine or lysine. Methylation of histones can provide either repressive or activating signals depending on the sites methylated and also the state of methylation (Bannister and Kouzarides 2005; Margueron et al. 2005). Lysine residues can be mono-, di- or tri-methylated, while arginine residues can be either mono-methylated and symmetrically or asymmetrically dimethylated (Fig. 2). HMTases responsible for these modifications show preference for substrate and variation in the state of methylation that is generated, creating specific histone methylation patterns for distinct functional outcomes (Bottomley 2004; Wang et al. 2003). Further, distinct families of proteins have been identified that specifically bind methylated histone lysine residues via recognition modules including the chromodomain, tudor domain, plant homeodomain finger (PHD) and WD40-repeat domain. The following discussion focuses on histone H3 methylation at lysine residues K4, K9 and K27.

Mammalian euchromatin is characterized by low level H3K4 dimethylation and trimethylation with peaks occurring at the 5′ region of some transcribed genes and also within the coding region of some genes (Francis et al. 2005; Litt et al. 2001a; Schneider et al. 2004; Valls et al. 2005). Several H3K4-specific HMTases have been identified including the mammalian proteins SET1/ASH2, SET7/9, MLL1/ALL1, MLL2/HRX2, MLL3/HALR and SMYD3. Each of these enzymes has been found in discreet multi-molecule complexes and may differentially catalyse mono- di- or trimethylation of H3K4 (Hamamoto et al. 2004; Kim and Buratowski 2009; Wysocka et al. 2005). H3K4 methylation promotes gene activity by creating a

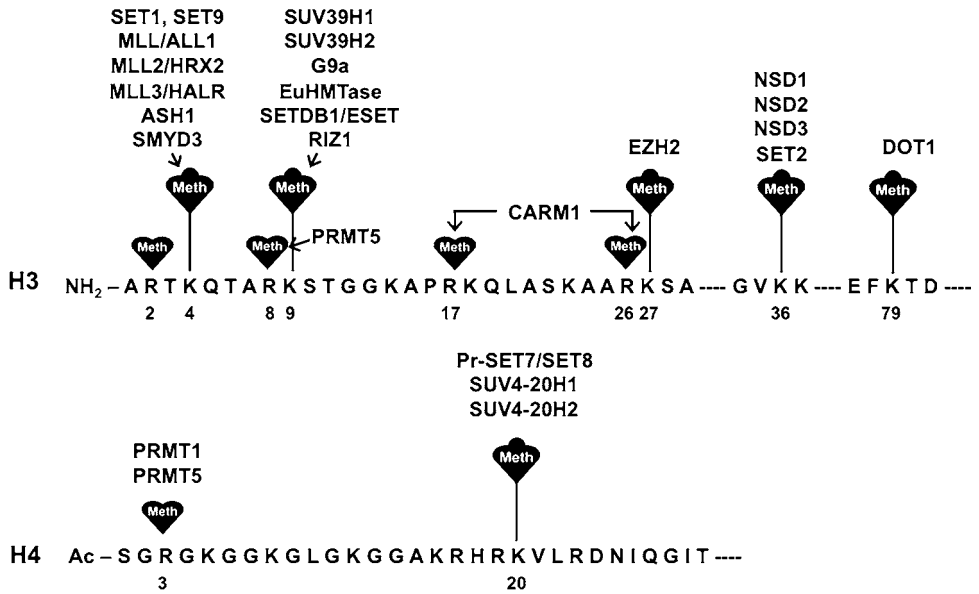

Fig. 2 Histone H3 and H4 methylation target sites, HMTase specificities and protein recognition domains. The amino acid sequence of H3 and H4 amino-terminal tails with extensions into the core (*dashed lines*) is shown with potential methylation sites numbered below—motifs representing arginine and lysine methylation are above sequence as per the legend. Human enzymes that can catalyse the specific modification are shown below the residue position. Protein domains that serve as receptors for each of the modifications are illustrated above the respective modification and are classed as chromodomains or tudor domains as indicated in legend. Examples of proteins containing specific recognition domains are indicated: CHD represents the chromodomain-helicase-DNA-binding domain of CHD1; HP1 proteins contain a common chromodomain; PcG represents polycomb protein chromodomains; 1 (p53 binding protein1) tudor domain; Cut5-repeat-binding protein 2 (*CRB2*) tudor domain

binding site for co-activators containing chromodomains that specifically bind this modification such as CHD1 (Flanagan et al. 2005; Sims et al. 2005). This protein had previously been identified as a transcriptional co-activator possessing ATPase and helicase domains similar to SWI/SNF members (Delmas et al. 1993). Consistent with H3K4 methylation stimulating gene expression, a class of co-activators including CHD1 (Lusser et al. 2005), specifically binds methylated H3K4, while a co-repressor complex has been identified which targets H3K4 demethylase activity to repressed promoters (Lee et al. 2005). In addition, components of the basal transcriptional machinery (Kanno et al. 2004; van Ingen et al. 2008) bind H3K4 residues to promote gene activation. Finally, H3K4 HMTase enzymes co-localise with RNA polII (Hamamoto et al. 2004; Milne et al. 2005), while depletion of H3K4 HMTase correlates with an aberrant distribution of RNA polII (Francis et al. 2005; Guenther et al. 2005).

Methylation of H3K9 is associated with a repressive chromatin environment and is enriched in constitutive heterochromatin, silent euchromatin and the inactivated X-chromosome (Cao and Zhang 2004; Peters et al. 2002; Roopra et al. 2004). Mammalian H3K9-specific HMTase enzymes include SUV39H1, SUV39H2, G9a, ESET/SETDB1 and Eu-HMTase1 (Rice et al. 2003; Wang et al. 2003). Methylation of H3K27 is also associated with repressive chromatin, and has been extensively studied in the context of ESC function, pluripotency and developmental gene regulation. The H3K27 methylation is catalysed by multi-molecular complexes referred to as polycomb repressive complexes (PRCs). While PRCs may be heterogenous in composition (Cao et al. 2008; Sarma et al. 2008) the EZH2 subunit has been identified as a key H3K27 HMTase and has been well characterized within the PRC2 complex (Simon and Lange 2008). EZH1 has also been identified in a non-canonical PRC2 complex and also posseses HMTase activity necessary for silencing a subset of developmental genes in ESCs (Shen et al. 2008).

Polycomb group (PcG) proteins containing the chromodomain play a key role in repression by binding methylated H3K27 (reviewed in Schuettengruber and Cavalli 2009), however, the mechanisms targeting PRC complexes and their specific effects during processes such as transcription and DNA replication are not well characterized (Francis et al. 2009; Margueron et al. 2009). Early studies demonstrated that, in vitro, PRCs promote chromatin compaction (Francis et al. 2004), inhibit chromatin remodeling (Francis et al. 2004), and block specific factors necessary for transcription (King et al. 2002). Moreover, H3K27 methylation enhanced binding of PRCs (Cao et al. 2002) while a PcG subunit (Enhancer of Zeste, the mouse homolog of EZH1) was identified as the HMTase component of PRC2 (Kuzmichev et al. 2002). While these studies present a strong link between H3K27 methylation and

chromatin silencing, the mechanisms that underlie this remain elusive. Indeed, H3K27 is not strictly required for PRC1 binding suggesting that alternative mechanisms may coordinate PRC distribution (Muller and Verrijzer 2009). Genome-wide studies of histone modifications and PRC occupancy in mammalian cells suggest that the underlying DNA sequence context plays a role in PRC recruitment, however, consensus binding sequences are not well defined (Ku et al. 2008). Furthermore, non-coding RNAs have been implicated in regulating PcG gene silencing (Grimaud et al. 2006; Moazed 2009). Interestingly, it has been suggested that chromatin marks important for maintaining pluripotency in embryonic stem cells, such as H3K27 methylation, may function in a distinct manner during later stages of development (Chi and Bernstein 2009). Indeed supporting this view, a unique PRC complex, designated PRC4, has been identified in embryonic stem cells and cancer cells, but is not detected in normal differentiated mammalian tissue (Kuzmichev et al. 2005). Thus, while H3K27 methylation and PRC recruitment are common events in developmental gene-silencing, the molecular mechanisms which establish or maintain these states are poorly understood.

Since histone methylation is a robust modification due to the high thermodynamic stability of the N–CH_3 bond, the mechanisms underlying histone demethylation remained contentious (Bannister et al. 2002) until the identification of the enzyme LSD1/KDM1 as a bona fide histone demethylase by Shi and co-workers (2004). Interestingly, LSD1 was found to demethylate mono- and di-methylated histone H3 at lysine positions H3K4 or H3K9. LSD1 may act as a co-repressor by demethylating H3K4 mono- and di-methyl groups thereby removing activating histone modifications (Shi et al. 2004). This activity is directed by accessory factors that make up the CoREST complex (Shi et al. 2005). In contrast, LSD1 functions as a co-activator when it is recruited by activated androgen receptors to androgen response elements. In this context LSD1 shows demethylase activity directed at repressive H3K9 mono- and dimethyl groups on local nucleosomes (Metzger et al. 2005). Therefore, like many chromatin regulatory enzymes, the activity of LSD1 is critically regulated by binding partners. Another class of histone demethylase enzymes containing the Jumonji domain (Jmjc) has now been identified, which include at least 15 distinct proteins that have been reported to demethylate specific lysine or arginine histone H3 residues (Cloos et al. 2008). Importantly, distinct groups of Jmjc enzymes catalyse lysine demethylation of H3K4 mono- di- or tri-methyl groups (JARID1B/PLU1, JARID1C/SMCX/KDM5C, JARID1D/SMCY/KDM5D, JARID1A/RBP2 and FBXL10/JHDM1b/KDM2B), H3K9 mono- di- or tri-methyl groups (JMD2A/JHDM3A/KDM4A, JMJD2C/GASC1/KDM4B, JMJD2D/KDM4D, JMJD1B/JHDM2b/KDM3B and JMJD1A/JHDM2a/KDM3A) or H3K27 mono- di- or tri-methyl groups (JMJD3/KDM6B and UTX/KDM6A). Therefore, histone demethylase enzymes comprise a large group of proteins which play a key role in the dynamic regulation of chromatin structure (Huang et al. 2006; Shi and Whetstine 2007; Whetstine et al. 2006).

Differentiation regulated histone methylation

Like histone H3 acetylation, H3K4 methylation is also enriched within active chromatin. For example, H3K4 dimethylated nucleosomes are located within the transcribed region, at transcriptional start sites (Liang et al. 2004) and/or promoter sequences (Adachi and Rothenberg 2005) of active genes as well as developmental genes poised for activation (Bernstein et al. 2006; Musri et al. 2006). It has been suggested that H3K4 methylation of nucleosomes at core promoter regions, may participate in targeting RNA polII to transcriptional start sites (Guenther et al. 2005; Hampsey and Reinberg 2003).

Histone modifications at *β-globin* regulatory regions during erythroid differentiation have been characterised in detail—in this model of developmental gene activation histone H3 K4 dimethylation is observed prior to transcription (Bottardi et al. 2003; Kim and Dean 2004; Levings et al. 2006). Similarly, H3K4 dimethylation has been observed at the *Il-2* and *Il-4-Il13* loci during T-lymphopoiesis (Adachi and Rothenberg 2005; Koyanagi et al. 2005) and the *apM1* promoter during adipogenesis (Musri et al. 2006) at developmental stages prior to initiation of expression and these marks persist after gene activation. While gene-specific studies have provided evidence of a functional link between histone H3K4 methylation and transcription, genome-wide analyses suggest that many genomic regions retain this modification both in embryonic and somatic cells independent of transcriptional activation (Guenther et al. 2007). Methylation of H3K4 at TSSs and transcription initiation does not strictly correlate with efficient production of full length transcripts, although, clusters of transcriptionally active cell-specific genes do show differential histone H3K4 tri-methylation at TSSs in differentiated cells compared to embryonic and silenced somatic cells (Guenther et al. 2007).

A genome-wide study examining histone H3K4 trimethylation and H3K27 trimethylation in naïve and memory T cells provided evidence that the combination of these PTMs was important in regulating the transcriptional responsiveness of genes important for CD8(+) T cell function (Araki et al. 2009). H3K4 trimethylation marks were associated with active genes differentially in long term, self renewing CD8-positive memory T cells and the short lived effector T cells. Interestingly, a subset of

"poised" genes were found to have higher levels of H3K4 trimethylation in memory T cells compared to naïve T cells. This sub-set of genes could be rapidly activated in memory T cells to facilitate the transition from resting to the activated state upon stimulation. On the other hand a sub-set of genes showed low level H3K4 methylation but were transcriptionally active in memory T cells compared to naïve T cells. The expression of this sub-set of genes was, however, associated with enhanced H3K9 acetylation in memory T cells. A global analysis of histone PTMs examining distinct subsets of CD4-positive T cells also revealed high level of H3K4 trimethylation correlating with actively transcribed loci in the different T cell subsets (Wei et al. 2009). Therefore, H3K4 methylation and expression of a subset of genes may be tightly associated with differentiation, while other genes retain this mark irrespective of transcriptional status and may function to poise genes for expression in response to external stimuli.

Genome-wide analyses of pluripotent embryonic cells have identified widespread regions containing both H3K4 trimethylation and H3K27 trimethylation (Bernstein et al. 2006; Ku et al. 2008). Termed "bivalent domains" due to the concomitant activating and repressive histone signatures, these histone modification patterns appear to support low level transcription. Bivalent domains may resolve at cell- and stage restriced loci during differentiation such that either H3K27 or H3K4 methylation persist at either silenced or transcriptionally active genomic regions respectively (Barski et al. 2007; Lee et al. 2006; Lim et al. 2009a; Mikkelsen et al. 2007; Sanz et al. 2008; Wang et al. 2008). Furthermore a study examining both H3K4 and H3K27 trimethylation in distinct subsets of CD4-postive T cells demonstrated H3K27 trimethylation at loci encoding master regulators of ESC function (e.g. *Nanog* and *Oct4*) or other cells fates (e.g the myogenic regulator, *MyoD* and a key B-cell specifc regulator, *Pax5*) (Wei et al. 2009). On the other hand, bivalent H3K4 and H3K27 trimethylation was observed at several loci considered master regulators of distinct CD4-positive subsets (e.g. *Tbx21* and *Gata3*). The authors speculate that these bivalent marks allow for plasticity in the CD4 positive cell phenotype in contrast to H3K27 methylation alone, which is observed at permanently silenced genes (Wei et al. 2009).

Changes in the distribution and dynamics of PRCs during embryonic differentiation (Ren et al. 2008) which may be targeted by non-coding RNAs (Simon and Kingston 2009) suggest a mechanistic link between the dynamic shift in histone the bivalent H3K4/K27 marks during differentiation and these non-coding RNAs. Indeed, while much is known about RNA-mediated gene silencing in plants (Preuss et al. 2008) and how this is directed by DNA methylation (He et al. 2009; Lister et al. 2008), relatively less is known about this in mammalian systems (Dinger et al. 2008; Franchina and Kay 2000). The recent identification of wide-spread non-CpG methylation in human embryonic stem cells compared to differentiated cells (Lister et al. 2009) may reflect a functional link between these previously poorly explored epigenetic modifications, PRC regulation and histone lysine methylation during ESC differentiation.

Histone H3K9 trimethylation is associated with silencing repetitive elements at centromeres, transposons and tandem repeats and may also play a role in transcriptional regulation (Peters et al. 2003; Rice et al. 2003). Methylation of H3K9 also plays a key role in silencing lineage specific genes during early embryogenesis (Tachibana et al. 2002, 2005). The functional relevance of H3K9 methyltransferase enzymes during development is evidenced by the crucial role of a key HMTase, G9a, during embryonic development (Tachibana et al. 2002). How this enzyme functions during differentiation is complex, for example, conditional knockout of G9a in lymphocytes shows that defects in H3K9 methylation perturbs later stages of B cell differentiation despite normal early development of precursor B cells (Thomas et al. 2008). Furthermore, a recent report suggests that G9a can function as a co-activator in some contexts independent of its HMTase activity (Chaturvedi et al. 2009). Thus, H3K9 methylation is an important pathway for silencing lineage-specific genes during development and may play a distinct role during later stages of differentiation. Surprisingly, H3K9 trimethylation has been observed at a subset of active promoters (Mikkelsen et al. 2007) as well as within the transcribed region of active genes (Vakoc et al. 2005, 2006) in mammalian cells. Also, H3K9 monomethylation has been observed at a subset of active enhancers (Wang et al. 2008), however, the relevance of these PTMs to transcriptional regulation is unknown.

The methylation of histone lysine residues is clearly a highly regulated process during differentiation. The extent of methylation and the position of the modified histone H3 lysine residues throughout the genome are precisely orchestrated as cells develop to enable cell- and stage-restricted transcription. Histone methylation is regulated by HMTase and histone demethylase enzymes, which are directed to genomic target sites by TF complexes containing these activities. Recent studies suggest that distinct combinations of histone methylations mark distinct regulatory elements differentially during development and gene activation (Heintzman et al. 2007; Wang et al. 2008). Genome-wide analyses of histone methylation states and transcription during differentiation of a range of cell types will provide valuable information to determine the global significance of each modification state in question. It will also be important to grasp how histone

methylation patterns are established and/or maintained by gene-specific TFs and/or non-coding RNAs during differentiation and how this relates to controlling the activity of the general TFs or alternatively, controlling chromatin compaction.

Histone phosphorylation

The regulation of cellular processes pertaining to the spatial and temporal acetylation/deacetylation and methylation of histones and nuclear histone-like proteins creates additional complexity to regulatory networks. Add to this histone phosphorylation, which is also linked to the regulation of chromatin structure and function associated with differentiation. Among the varied list of histone modifications, already described above, the array of histone modifications can be further complicated by the role histone phosphorylation plays in processes such as transcription, DNA repair, apoptosis and chromosome condensation (Cheung et al. 2000). In a similar fashion to acetylation, the phosphorylation of histones is able to act as an on/off switch in regulating the interaction between histones and DNA and between histones and accessory proteins that interact with the nucleosome.

Phosphorylation of histones contributes to chromatin function and structural architecture in most cases via serine or threonine phosphorylation of histone tails. Phosphorylation of N-terminal histone tails and the role they play in chromatin structure and function have been comprehensively reviewed elsewhere (for reviews see Cheung et al. 2000; Fillingham and Greenblatt 2008; Peterson and Laniel 2004). In addition to the well documented processes regulated by N-terminal histone tail phosphorylation, there is a growing body of evidence to suggest that histone phosphorylation within the core and at the C-terminus sequence of some histones, including in once instance the histidine phosphorylation on histone H4, is also responsible for alterations in chromatin structure, function and cell regulation. It is these 'less' commonly reported non N-terminal tail histone phosphorylation events that will be addressed below with respect to the control of various cellular processes such as differentiation (Dawson et al. 2009), apoptosis and DNA repair (Solier and Pommier 2009). The currently reported sites of all known histone phosphorylations are illustrated in Fig. 3. This list is by no means complete as other sites are likely to be discovered and involved in regulatory mechanisms not mentioned in this article. Although discussed in isolation, many of the phosphorylation events described below work in concert with other multiple histone modifications previously mentioned above as part of the histone code (Cheung et al. 2000).

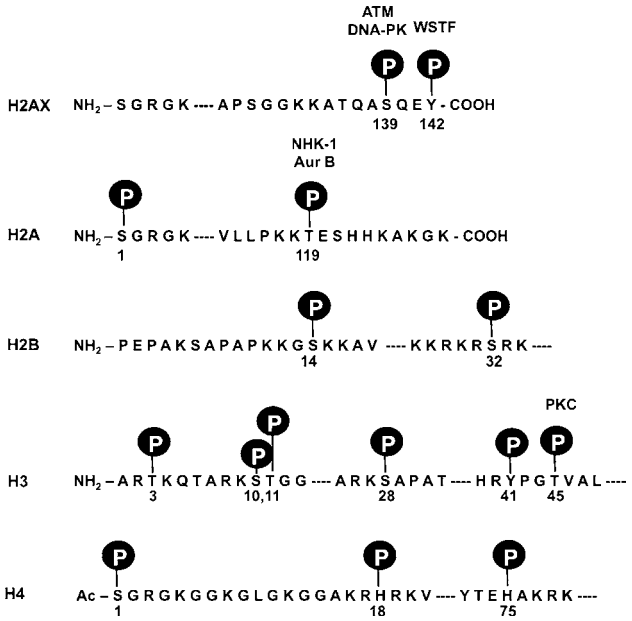

Fig. 3 Histone H2A, H2AX, H2B, H3 and H4 phosphorylation target sites. The amino acid sequence of histones amino-terminal tails with extensions into the core (*dashed lines*) is shown with potential phosphorylation sites numbered below. Putative kinases are shown above the residue position for core and C-terminal phosphorylations. *T119 is from the Drosophila H2A amino acid sequence (Brittle et al. 2007)

H2A phosphorylation

The phosphorylation at the C-terminus of histone H2A occurs on residue threonine 119 (T119) (Fig. 3). One mechanism thought to involve the phosphorylation of T119 is the regulation of chromatin structure and function during mitosis. Using antibodies specific for the T119 phosphorylated form of H2A, (Brittle et al. 2007) were able to demonstrate that in the developing *Drosophila* embryo, T119 phosphorylation occurs in during mitosis but not in S-phase of the cell cycle. The cognate kinase NHK-1 (Nucleosomal histone kinase-1) phosphorylates T119 in chromatin but not the soluble pool of core histones. It appears that NHK-1 has a high affinity for chromatin and selectively phosphorylates H2A (not the H2Av or H2Ax variants of the protein) only when it is in a nucleosomal framework. The apparent absence of T119 phosphorylation of free H2A in solution is suggestive of the important role T119 phosphorylation has in regulating the chromatin architecture during mitosis. Whilst NHK-1 phosphorylates T119 throughout the chromatin it is not responsible for the abundance of T119 phosphorylation in the centromeric regions during mitosis. That role appears to fall to the Aurora B kinase complex.

Aside from NHK-1 and the Aurora B kinase complex it was found that in the *Drosophila* embryo, there are several other key players that control the status of T119

phosphorylation during the cell cycle. There also appears to be a role for Polo kinase to down-regulate H2A phosphorylation on the chromosomal arms during mitosis (Brittle et al. 2007). Through epistatic analysis, the Polo kinase activity appears to temporally occur upstream of NHK-1 activity. Thus T119 phosphorylation of H2A throughout the chromosome and its role in regulating chromatin structure and function is thought to be both spatially and temporally controlled by the concerted action of several protein kinases during mitosis.

H2AX phosphorylation

Post-translational modification of the H2AX isoform of H2A has been implicated in the genomic response to DNA damage. One response to this damage is the phosphorylation of H2AX. Phosphorylation of H2AX has been shown to accumulate at sites of DNA double-stranded breaks, leading to a restructure of the chromatin and assisting in the recruitment of DNA repair and signaling factors. The site of H2AX phosphorylation in response to double stranded breaks occurs at serine 139 (Solier and Pommier 2009). However, there is also emerging evidence that tyrosine 142 is also a possible site of phosphorylation (Cook et al. 2009; Xiao et al. 2009). In addition to phosphorylation in response to double stranded DNA breaks, S139 phosphorylation is also implicated early in apoptosis when induced by death receptor activation. Temporally, evidence using phospho-S139 specific antibodies suggests that the S139 phosphorylation is induced in the latter stages of apoptosis (Solier and Pommier 2009).

The S139 phosphorylation that occurs in response to DNA damage is mediated by the PI3-like kinases, ATM and DNA-PK. However, both ATM and DNA-PK independent phosphorylation of S139 have also been reported in the skin of mice lacking these kinases (Koike et al. 2008a, b). In the case where S139 phosphorylation is implicated in apoptosis, it is thought that DNA-PK is the cognate kinase responsible for its phosphorylation (Solier and Pommier 2009) whereas ATM is thought to be responsible for S139 phosphorylation in instances of DNA damage.

The phosphorylated serine in H2AX is part of a conserved motif (ASQE found in the carboxyl terminus of yeast H2A1 and H2A2 variants and H2AX in the mammalian cells) that is rapidly phosphorylated upon exposure to DNA-damaging agents (Downs et al. 2000; Rogakou et al. 2000). In yeast, the PI3-like kinase Mec1 (ATR is the mammalian homologue) is apparently required for efficient non-homologous end-joining repair of DNA. Thus the phosphorylation of S139 makes a vital contribution in mediating an alteration of chromatin structure, which ultimately facilitates repair of the DNA (Grant 2001).

The other more recently reported site of H2AX phosphorylation can be found on Y142 and is thought to be important in the delineation of either a repair/survival or apoptotic pathway after DNA damage (Cook et al. 2009; Xiao et al. 2009). Y142 phosphorylation is mediated by WSTF, a subunit of WICH complex. The WSTF subunit contains a novel kinase domain at its N-terminus that appears to target H2AX on Y142. Thus Y142 and S139 phosphorylation together may play a role in switching the pathway between cell life and death. The capacity for phosphorylation at both positions reinforces the whole concept of the histone code and its epigenetic control of molecular pathways in the cell.

H2B phosphorylation

There are no reports of core or c-terminal histone H2B phosphorylation.

H3 phosphorylation

Histone H3 is known to undergo phosphorylation at multiple sites (see Fig. 3 for reference) but most of these are found in the N-terminal tail of the protein. The phosphorylation most distal to the N-terminus is at threonine 45. Like H2A/X, phosphorylation of histone H3 at T45 is also known to occur after activation of DNA-damage signaling pathways (Hurd et al. 2009).

In apoptotic cells T45 phosphorylation occurs at a time when the DNA strand is nicked.

An example of this can be found in cultured human neutrophils. Upon being freshly isolated, these cells contain very little phosphorylated T45, however the phosphorylation of T45 increases rapidly after only 20 h in culture (Hurd et al. 2009). The timing of T45 phosphorylation closely parallels the activation of the caspase 3 which further supports the hypothesis of a temporal association between histone H3 phosphorylation and apoptosis.

Both in vivo and in vitro, protein kinase C has been identified as the cognate kinase responsible for the phosphorylation of T45 (Hurd et al. 2009). Protein kinase C plays a pivotal role in many cellular signaling cascades with apoptosis being just one of them. Hence is not surprising to find yet another example of histone phosphorylation playing a role in apoptotic pathways. Given its structural position in the nucleosome (Luger et al. 1997a, b), it is possible that T45 phosphorylation induces structural changes which exposes the DNA and facilitates DNA nicking and/or the fragmentation associated with apoptosis.

Moving slightly closer to the N-terminus we also find phosphorylation of Y41 on histone H3 that appears to play a role in differentiation associated with haematopoiesis. This more recently discovered phosphorylation appears to

be implicated in normal haematopoiesis and leukaemia (Dawson et al. 2009). The cognate kinase responsible for this phosphorylation event appears to be the non-receptor tyrosine kinase JAK2. In the nucleus, JAK2 directly phosphorylates Y41 thus preventing the binding of heterochromatin protein 1alpha. Dawson et al. demonstrates the significance of Y41 phosphorylation when JAK2 is inhibited in leukaemic cells. This inhibition leads to both a decrease in Y41 phosphorylation and a decrease in the expression of the haematopoietic oncogene lmo2. The subsequent decrease in Y41 phosphorylation caused a concomitant increase in the binding of heterochromatin protein 1alpha to the Y41 region. This new role for JAK2 establishes a novel link between the JAK2 and Imo2 genes in haematopoiesis and leukaemia.

H4 phosphorylation

In addition to phosphorylation of serine 1, histone H4 has the unusual honour of also being phosphorylated on one of two histidine residues, H18 and H75 (Besant et al. 2003). In keeping with the focus on non N-terminal tail phosphorylation, only H75 will be highlighted. Unlike the more common serine, threonine and tyrosine phosphorylation, histidine is capable of being phosphorylated as one of two possible isomeric forms (1-phosphohistidine or 3-phosphohistidine) owing to the nature of the available amino groups on the imidazole moiety (for a review see (Attwood et al. 2007)).

Although there is overwhelming evidence of histidine phosphorylation of histone H4, to date, the kinase responsible has yet to be definitively identified. Partially purified fractions from various sources are known to contain histidine kinases, the best of these being a well characterised yeast histone H4 kinase (Huang et al. 1991; Wei and Matthews 1990) along with one from *P. polycephalum* (Huebner and Matthews 1985). Both of these examples are known phosphorylate histone H4 on H75 to form the 1-phosphohistidine isomer. In addition to these two examples, there are also earlier reports of mammalian histidine kinase activity that are less specific with respect to which phosphohistidine isomer is produced (Chen et al. 1977, 1974; Smtih et al. 1973).

Mammalian histone H4 histidine kinase activity has been reported from various types of tissue/cells. Evidence for this includes [31P]-NMR data from which a histone H4 histidine kinase was isolated from Walker-256 carcinosarcoma cells that phosphorylates both H18 and H75 on histone H4, forming 3-phosphohistidine. Similarly, an enzyme identified in regenerating rat liver was also shown to phosphorylate both histidine residues but this time to form 1-phosphohistidine (Fujitaki et al. 1981; Smith et al. 1974). The functional significance of the formation of the different isoforms of phosphohistidine is not known. What is known is that the lability of 1-phosphohistidine is much greater than 3-phosphohistidine for the free phosphoamino acids (Attwood et al. 2007). If the lability for each respective isomer remains true in the context of a protein histidine phosphorylation, then the type of isomer employed may regulate the timing of the phosphorylation/dephosphorylation event with respect to its associated function.

The original report of histone H4 kinase activity sourced from regenerating rat liver, also presents evidence to suggest that are histone histidine kinases present in the nuclei of cells from other rat tissue sources (Smtih, et al., 1973). Screening of rat tissues indicates that proliferating thymocyte nuclei were also a rich source of histone H4 histidine kinase activity. Besant and Attwood (Besant and Attwood, 2000) partially purified histone H4 kinase activity from porcine thymus and the preparation was shown to contain up to four putative histone H4 histidine kinases with apparent molecular weights in the range of 34–41 kDa. The biological function of these histone H4 histidine kinases remains to be determined, however their presence in tissues such as Walker carcinosarcoma cells, thymus, regenerating liver, foetal liver, liver progenitor cells and hepatocellular carcinoma (Tan et al. 2004) suggests that they may play a role in cellular proliferation and differentiation. Although this is speculative, the correlation between histone phosphorylation and cell division, as well as chromatin condensation associated with entry into mitosis has been well documented (Bradbury 1992; Gurley et al. 1978; Mizzen et al. 1998).

H75 of histone H4 has been determined to be located close to the DNA binding site within the nucleosome core in calf thymus. The imidazole ring of H75 also forms a hydrogen bond with E90 of histone 2B within the intact nucleosome core, thus helping to stabilise the histone octamer (Luger et al. 1997a, b). Phosphorylation of this histidine could thus result in the destabilisation of the nucleosome structure thus assisting in the replication process of the DNA during the cellular proliferation with which it is associated. Contrary to this supposition is the evidence of (Wei et al. 1989) who showed that in *P. polycephalum,* histone H4 in nucleosome core particles is not a substrate for the cognate histidine kinase activity. So what is the histidine phosphorylation state on newly synthesized histones required for packing up DNA during replication? Chen and co-workers (1977) presents evidence that newly synthesised histone H4 in regenerating rat liver cells is not phosphorylated on histidine. Therefore, it may be possible that phosphorylation of pre-existing histone H4 at the time the histones are displaced from DNA during replication, may disrupt histone–histone interactions and binding to DNA to prevent pre-existing histones from

prematurely forming nucleosome complexes during DNA synthesis (Chen et al. 1977; Wei et al. 1989).

Conclusion

The enzymes which covalently modify histones or TF complexes, which recognize these modifications, function at distinct stages to activate developmentally regulated genes. It is now clear that many chromatin-associated TF complexes are also regulated by PTMs including acetylation, methylation and phosphorylation, and these modifications in turn regulate histone modifications. Thus, studies examining mammalian differentiation support the "histone code hypothesis", however, the concepts underpinning this theory extend to include site specific PTMs to non-histonal nuclear proteins (Sims and Reinberg 2008).

The combination of modifications to histone and non-histonal proteins is extremely variable. Studies, which illuminate how distinct patterns of histone modifications are distributed throughout the genome, have provided unique insight into how these phenomena relate to gene expression. It is possible that common patterns of histone PTMs reflect co-localization of regulatory elements into shared nuclear subcompartments including "transcription factories" or constitutive heterochromatin (Osborne et al. 2004). This hypothesis implies that groups of genes or distant regulatory regions, are organized within the nucleus to enable co-regulation of differentially expressed genes during development. Accordingly, histone modifications would be similar within a cluster of co-regulated genes, but still allow for variation to fine-tune the timing or level of gene expression during differentiation. Indeed, diseases can arise when long-range interactions between regulatory regions are interrupted or when domain-wide histone PTMs develop abnormally (Kleinjan and Lettice 2008; Kleinjan and van Heyningen 2005). To gain a thorough understanding of differentiation-induced transcription, it will be necessary to determine how TFs orchestrate these changes to histones during differentiation of different cell-lineages and how this relates to the spatial re-organization of the genome during development. While there is little doubt that that these PTMs play a pivotal role in both regulation and chromatin architecture, it is the combined spatial and temporal changes of all histone PTM's that directs the symphony of epigenetic changes within the cell. We have attempted to highlight a few of the modifications that play crucial roles in regulating many of these biological processes. Further research into the complexities and fidelity of the entire network of changes and responses to histones and histone accessory proteins will no doubt reveal even more exciting discoveries.

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