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Review

Lateral Thinking: How Histone Modifications Regulate Gene Expression

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The DNA of each cell is wrapped around histone octamers, forming so-called 'nucleosomal core particles'. These histone proteins have tails that project from the nucleosome and many residues in these tails can be post-translationally modified, influencing all DNA-based processes, including chromatin compaction, nucleosome dynamics, and transcription. In contrast to those present in histone tails, modifications in the core regions of the histones had remained largely uncharacterised until recently, when some of these modifications began to be analysed in detail. Overall, recent work has shown that histone core modifications can not only directly regulate transcription, but also influence processes such as DNA repair, replication, stemness, and changes in cell state. In this review, we focus on the most recent developments in our understanding of histone modifications, particularly those on the lateral surface of the nucleosome. This region is in direct contact with the DNA and is formed by the histone cores. We suggest that these lateral surface modifications represent a key insight into chromatin regulation in the cell. Therefore, lateral surface modifications form a key area of interest and a focal point of ongoing study in epigenetics.

Trends

The globular domains of histones represent the emerging front of histone modification research.

Modifications in the globular domains of histones can directly affect transcription and nucleosome stability.

Many globular domain modifications also have roles in the DNA damage response, stemness, leukaemia and cell differentiation.

Novel modifications, such as arginine methylation, are also present in this region and can directly affect the compaction of the DNA coating the nucleosome.

The Nucleosome: How to Package a Genome

Each diploid human cell contains approximately 2 m of DNA [1], which must be accessed when needed, for instance for transcription or replication. This requires that the transcriptional or replication machinery get to the required genomic regions at the correct time, despite the generally highly compacted DNA within the nucleus of the cell. Early studies showed that DNA, despite its natural tendency to become disordered [1], is organised into tightly regulated structures [2,3]. This organisation begins with the wrapping of the DNA around an octameric protein complex, forming a so-called 'nucleosomal core particle' [4]. Each of these contains two of each core histone (H2A, H2B, H3, and H4 [5–7]) with 145–147 base pairs (bp) of DNA wound around it. A histone known as linker histone H1 is bound to the outside of the nucleosome core particle, forming a full nucleosome or chromatosome, and stabilising higher-order chromatin structures [4,8]. Nucleosomes are found every 200 ± 40 bp [9] and they form a characteristic 'beads on a string' structure with their coating DNA [10].

In general, nucleosomes impede transcription of the DNA [11,12]. They may do this by physical obstruction, as well as by bending the DNA, thus reducing its availability to transcription factors [5]. However, histones can also carry many post-translational modifications and these can influence chromatin compaction and accessibility in many different ways. These modifications include acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, ADP ribosylation, and deamination [13]. More recently, other modifications, such as propionylation and butyrylation, have been described [14]. So far, the best-studied modifications are those that

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Table 1. Histone Tail Modifications

Histone	Modification	Role	Refs
H2A	H2AS1P	Mitosis; chromatin assembly	[98]
	H2AK4/5ac	Transcriptional activation	[99]
	H2AK7ac	Transcriptional activation	[100]
	H2AK119P	Spermatogenesis	[101]
	H2AK119uq	Transcriptional repression	[102]
H2B	H2BS14P	Apoptosis	[103]
	H2BS33P	Transcriptional activation	[104]
	H2BK5ac	Transcriptional activation	[105]
	H2BK11/12ac	Transcriptional activation	[100]
	H2BK15/16ac	Transcriptional activation	[100]
	H2BK20ac	Transcriptional activation	[105]
	H2BK120uq	Spermatogenesis/meiosis	[101]
	H2BK123uq	Transcriptional activation	[106]
H3	H3K4me2	Permissive euchromatin	[107]
	H3K4me3	Transcriptional elongation; active euchromatin	[26,107–109]
	H3K9me3	Transcriptional repression; imprinting; DNA methylation	[26,110]
	H3R17me	Transcriptional activation	[111,112]
	H3K27me3	Transcriptional silencing; X-inactivation; bivalent genes/gene poising	[26]
	H3K36me3	Transcriptional elongation	[26]
	H3K4ac	Transcriptional activation	[109]
	H3K9ac	Histone deposition; transcriptional activation	[100]
	H3K14ac	Transcriptional activation; DNA repair	[26]
	H1K18ac	Transcriptional activation; DNA repair; DNA replication	[26]
	H3K23ac	Transcriptional activation; DNA repair	[26]
	H3K27ac	Transcriptional activation	
	H3T3P	Mitosis	
	H3S10P	Mitosis; meiosis; transcriptional activation	[110]
	H3T11/S28P	Mitosis	
H4	H4R3me	Transcriptional activation	[87]
	H4K20me1	Transcriptional silencing	[113]
	H4K20me3	Heterochromatin	[114]
	H4K5ac	Histone deposition; transcriptional activation; DNA repair	[100,115]
	H4K8ac	Transcriptional activation; DNA repair; transcriptional elongation	[100,115]
	H4K12ac	Histone deposition; telomeric silencing; transcriptional activation; DNA repair	[100,115]
	H4K16ac	Transcriptional activation; DNA repair	[16,100,115]
	H4S1P	Mitosis	[98]

occur on the N-terminal 'tail' regions of the histones, which project from the nucleosome and are accessible on its surface [5] (Table 1 and Figure 1). Some of the modifications in these tails can directly affect the interactions between nucleosomes. For example, the addition of acetyl moieties to lysine 16 of histone H4 (H4K16ac) has been shown to reduce chromatin compaction [15] and increase transcription both *in vitro* and *in vivo* [16]. Histone tail modifications can also do

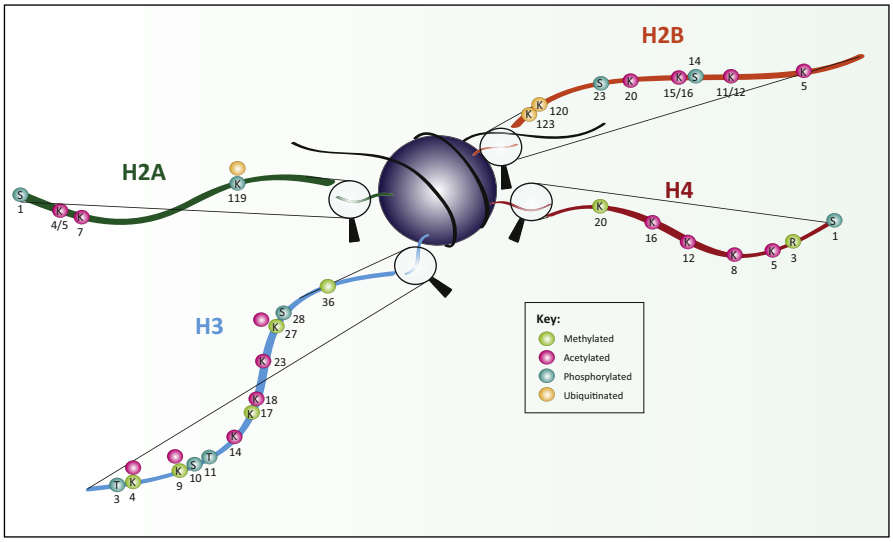


Figure 1. Schematic Showing Post-Translational Modifications of the Histone Tails. The location of each modification is shown in black and the amino acid modified at each position is also shown (K = lysine, R = arginine, S = serine, T = threonine). Colours depict how each residue is modified (green = methylated, pink = acetylated, turquoise = phosphorylated, beige = ubiquitinated).

the reverse and increase DNA compaction; for example, H4K20 di- and tri-methylation, which have been shown to enhance *in vitro* chromatin condensation [17]. However, this mode of action for histone tail modifications might not be the rule but rather the exception since these two modifications are the only histone tail modifications that have been shown to have a direct effect on chromatin architecture in *in vitro* assays.

In addition to these direct effects, histone modifications usually act indirectly and recruit effector proteins to activate downstream signalling [18], block the access of remodelling complexes [19], or influence the recruitment of chromatin modifiers and transcription factors [20,21].

New histone tail modifications are still being discovered, adding to our knowledge of how post-translational modifications can respond to and influence gene transcription and chromatin function. However, histone tails can be completely deleted and this has no major effect on nucleosome stability [22]. Therefore, tail modifications, despite their possible effects on chromatin action, are not essential for nucleosomal integrity.

As well as the tails, other regions of the histone can also be modified. The central globular domains of the histones, which together form the core of the nucleosome, also contain a large number of modification sites (Table 2) [24,55]. Within this globular domain, the lateral surface, which is the outer region of the histone octamer and has direct contact with the DNA, is of particular interest. If the octamer were a jam jar, this lateral surface would be the label. Recently, mass spectrometry has enabled the identification of novel modifications in the globular domain, especially on its lateral surface [25,26]. Thus, we focus here on novel modifications within the globular domain. For modifications within the histone tails, we refer readers to recent reviews [24] (see Table 1 and Figure 1).

Globular Domain Modifications: H3K79, an Activator with a Role in Silencing

One of the first-studied modifications in the globular domain of histones was the methylation of K79 of H3 [26], a modification whose functions have not yet been fully resolved. This residue is

Table 2. Histone Globular Domain Modifications

Histone	Site	Modification	Refs
H2A	H2AK36	Acetylation	[24,96]
	H2AK99	Methylation	[24]
	H2AQ105	Methylation	[89]
	H2AK119	Acetylation	[24]
	H2AK119	Ubiquitylation	[102]
H2B	H2BK40	Methylation	[24]
	H2BK82	Acetylation	[24]
	H2BR96	Methylation	[105]
	H2BK105	Acetylation	[100]
	H2BK113	Acetylation	[100]
	H2BK117	Acetylation	[105]
H3	H3Y41	Phosphorylation	[91]
	H3R42	Methylation	[88]
	H3T45	Phosphorylation	[92]
	H3R53	Methylation	[24]
	H3K56	Acetylation	[64]
	H3K56	Methylation	[56]
	H3K64	Acetylation	[25]
	H3K64	Methylation	[81]
	H3K79	Methylation	[26–28]
	H3K115	Acetylation	[24]
	H3T118	Phosphorylation	[94–96]
	H3K122	Acetylation	[23]
H4	H4K31	Acetylation	[24]
	H4S47	Phosphorylation	[116]
	H4K59	Methylation	[96]
	H4K77	Acetylation	[24]
	H4K79	Acetylation	[117]
	H4K91	Acetylation	[117,118]
	H4R92	Methylation	[24]

found at the solvent-exposed faces of the histone octamer and, thus, is easily accessible on mononucleosomes (Figure 2). H3K79 mono-, di-, and tri-methylation was first identified in yeast [27,28]. The mutation of H3K79 to nonmethylatable residues causes defects in telomeric silencing in yeast [29]. It is likely that it has a similar effect in mammalian cells because mouse embryonic stem cells (ESCs) lacking all methylated H3K79 have elongated telomeres [30]. However, despite its repressive effect on telomeres, H3K79 methylation in all its states is correlated with active gene expression in many cell systems (Figure 3) [31–36].

Disruptor of telomeric silencing 1p (Dot1p) was identified as the enzyme responsible for H3K79 methylation in *Saccharomyces cerevisiae* [27–29]. Dot1p requires intact nucleosomes to methylate H3K79 [27] and subsequent studies indicated that it relies on the presence of H4 [37,38] and H2BK123Ub [34,38] to recognise its substrate. Ninety percent of yeast H3K79 is methylated [27], whereas the levels are lower in mammals. DOT1L is the enzyme responsible for this

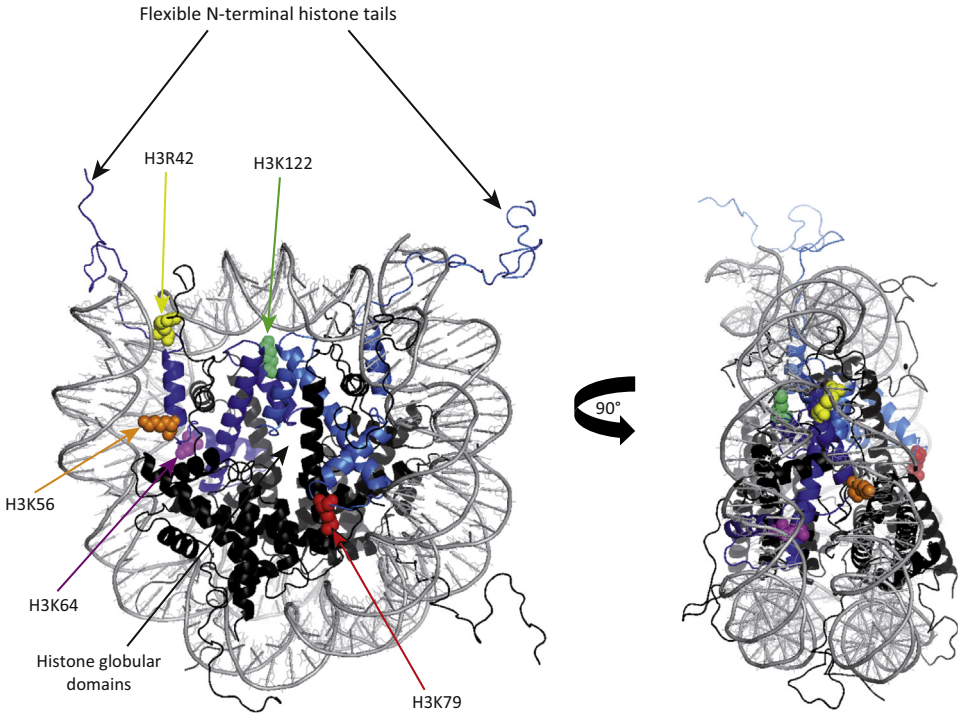
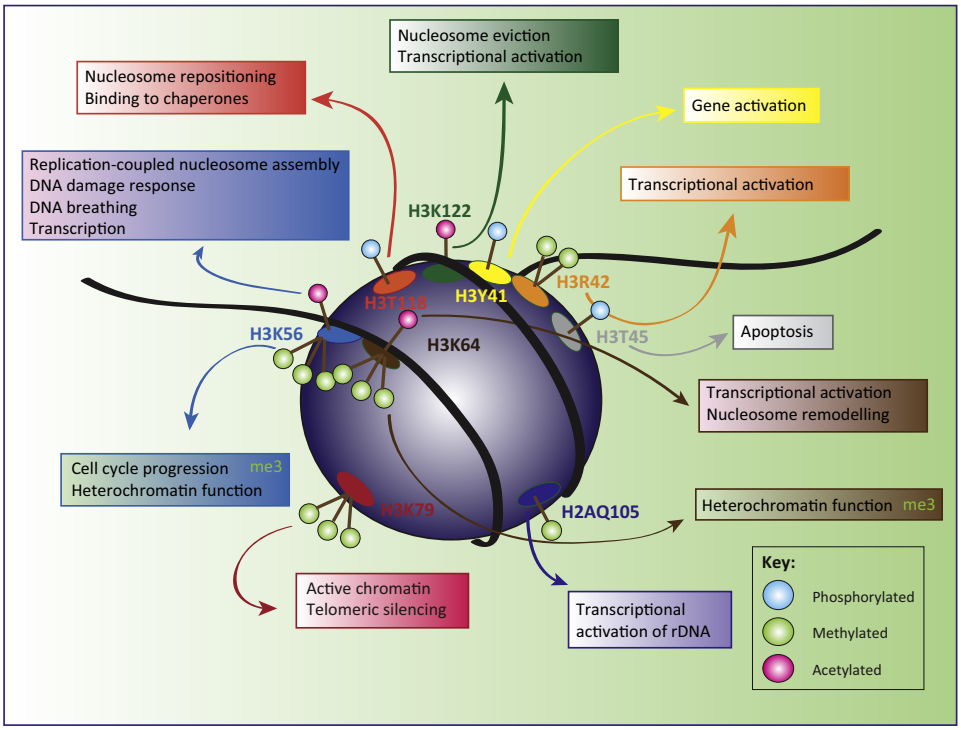


Figure 2. Location of Histone Globular Domain Modifications. Crystal structure of a histone octamer with H3 histones shown in light blue and purple. DNA is depicted in grey. H3K79 is indicated in red and is located on the solvent-exposed faces of the octamer. H3R42 (yellow), H3K56 (orange), H3K64 (pink), and H3K122 (light green) are shown on the lateral surface of the histone. Face-on and side views are shown. Note that H3R42, H3K56, H3K64, and H3K122 are located directly underneath the DNA.

mark in mammals. Mouse embryonic fibroblasts (MEFs) with mutant DOT1L lack all H3K79me2/3, indicating that it may be the only enzyme responsible for their deposition [36].

The role of H3K79me3 in transcription is well described. H3K79me3 is found on the transcriptional start site of active genes [36,39], whereas H3K79me1 extends to regions over 30 kb from active genes [36]. As well as being potentially involved in transcription, H3K79me1 is characteristic of a 'poised state' because it is found on genes that have both H3K4me3 and H3K27me3 marks in mouse ESCs (ESCs) [36,40]. It also marks distant regulatory elements [36]. Thus, it is found at both active genes and those that are poised for activation. H3K79me2 is enriched at transcribed regions in *Drosophila melanogaster* and absent from heterochromatic regions [35]. Despite its role in delineating active genes, H3K79me2 opposes the deposition of H4K16ac, one of the only marks that has a direct impact on chromatin structure and transcription [16,34]. H3K79me1/2/3 levels correlate with transcript abundance, again indicating that it is an active mark [35,36]. Hypomethylated H3K79 is found on heterochromatic regions in *S. cerevisiae* [34], supporting the idea that H3K79 methylation occurs on active regions. Furthermore, in mammals, H3K79 methylation levels change during gene induction and differentiation in multiple cell systems [34,36,41,42] and have been found to be associated with an active chromatin state.

Conversely, other groups have reported enrichment of H3K79me3 on transcriptionally repressed genomic regions. For example, H3K79me3 was described to be enriched on DNA methylated alleles in imprinted regions, whereas me1/2 was preferentially found on non-DNA-methylated alleles [43]. This would indicate the reverse of other studies, namely that H3K79me3 is found on repressed regions, whereas me1/2 is found on active regions. Mutating



Trends in Genetics

Figure 3. Schematic of a Nucleosome with Its Coating DNA, Detailing the Locations and Functions of Key Modifications within the Globular Domains of the Histones. Methyl marks are shown in light green, acetyl marks in pink, and phosphorylated residues in light blue.

the CCCTC-binding factor (CTCF) sites of the insulin-like growth factor (Igf)/H19 locus, causing aberrant bi-allelic transcription of Igf and H19, increased overall H3K79me1/2 levels on the locus. By contrast, H3K79me3 was redistributed to both alleles despite the fact that overall levels did not increase.

The role of H3K79 in gene regulation may influence the epigenetic reprogramming that occurs during early development. H3K79me3 is enriched on pericentromeric heterochromatic regions in mouse oocytes, whereas H3K79me2 is more diffusely located [44]. Both marks are removed after fertilisation. Therefore, these marks appear to disappear during the epigenetic erasure that occurs in development. Thus, H3K79 methylation could be required for differentiation. H3K79me2 is present on the promoters of pluripotency-associated genes, such as Octamer-Binding Protein 4 (*Oct4*) and Sex determining region Y (SRY) box 2 (*Sox2*) in mESCs [32]. ESCs can be maintained after Dot1L knockdown (KD), despite an increased propensity to become aneuploid and with slightly slower proliferation rates than controls [32]. However, they undergo cell cycle arrest if induced to differentiate under KD conditions [32]. Dot1L$^{-/-}$ teratomas have higher residual *Oct4* expression compared with controls [32], indicating slower differentiation. Similarly, Dot1L is required for embryonic development because *Dot1l* gene-targeted mice undergo developmental arrest at embryonic day (E)9.5 [30].

Similar results are seen during the reprogramming of human fibroblasts to induced pluripotent stem cells (iPSCs). The presence of methylated H3K79 appears to restrict reprogramming because the KD or inhibition of DOT1L, the H3K79 methyltransferase, increases reprogramming efficiency [45]. However, it could also enable reprogramming by selecting against incompletely reprogrammed intermediates because DOT1L inhibition compromises JF1 MEF viability [43].

During the reprogramming induced by somatic cell nuclear transfer (SCNT) into oocytes, H3K79 demethylation occurs in the somatic nucleus, linking the KD or inhibition of DOT1L to changes that occur during reprogramming [44]. Therefore, removing DOT1L could reduce H3K79 methylation levels, similar to what would happen during successful reprogramming. In conclusion, during both early embryonic development and *in vitro* reprogramming, H3K79 demethylation could be required for epigenetic reprogramming to occur.

H3K79me2 also has links with cancer. The inhibition of DOT1L inhibits breast cancer cell proliferation and metastasis [46]. H3K79me2 is found on many mixed lineage leukemia (MLL) target genes in MLL-induced leukaemia [41,42,47]. The inhibition of DOT1L erases this gene expression signature [47] and inhibits colony-forming ability [41], indicating that H3K79 methylation may be deposited on target genes as a consequence of leukaemic transcriptional alteration. DOT1L is thought to be recruited by ALL1-Fused Gene From Chromosome 9 Protein (AF9) [48], which binds acetylated H3K9, H3K18 and H3K27 marks [49]. The involvement of H3K79me2 in cancer could also be due to its role in the DNA damage response. H3K79me2 can recruit Tumor protein P53 binding protein 1 (TP53BP1) to double-stranded breaks [50], downstream of the H2BK123ub modification that occurs at these sites [51]. Therefore, the interplay between H3K79 methylation and other chromatin modifications in the vicinity, as well as its protein-binding partners, may influence the susceptibility of a cell to cancer and other cell state alterations.

Pushing Transcriptional Change: H3K122ac

H3K79 methylation is associated with regions of active or inactive transcription, depending on the extent of methylation and the cell system used and, so far, all evidence points to its rather indirect effects on chromatin function. However, other globular domain modifications can have direct effects on transcription. H3K115 and H3K122 were both identified as novel acetylation sites in the dyad axis region of the nucleosome (Figure 2) [52,53]. The dyad axis is the part of the lateral surface that is coated by only one fibre of DNA. Histone binding to the DNA is at its highest strength here [54]. Tropberger *et al.* found that H3K122ac co-localises with many marks characteristic of active genes (H3K4me3, H2A.Z, and H3.3) and enhancers (H3K4me1 and H3K27ac) [23]. Regions with H3K122ac marks are nucleosome poor and H3K122ac is also enriched around the transcriptional start site of genes, where nucleosomes would be displaced for transcription to occur. However, unlike H3K79 methylation, this is more than just correlative because H3K122ac promotes nucleosome eviction, increasing the access of the transcriptional machinery [23]. As a result, H3K122ac can have direct effects on transcription and its presence increases transcription in *in vitro* assays [23,55]. This was the first demonstration of a causative function for histone modifications in transcription. In support of this, yeast strains containing only nonacetylatable H3K122 are viable but the absence of H3K122ac significantly slows down gene induction [23]. Therefore, H3K122ac has a direct influence on transcription and is not merely a readout of gene activity (Figure 3).

Moreover, H3K122ac forms a novel mark with interesting kinetics upon gene induction. H3K122ac is deposited only 10 min after estrogen addition during estrogen-mediated gene activation, in contrast to H3K4me3, which is a classical mark of active genes and is only maximally deposited after 30–40 min. Therefore, such novel histone modifications can be more accurate reflections of gene state than classical marks. For the future, it will be important to understand the function of such modifications beyond transcriptional start sites.

Regulating Chromatin State with Two Opposing Modifications: H3K56 and H3K64

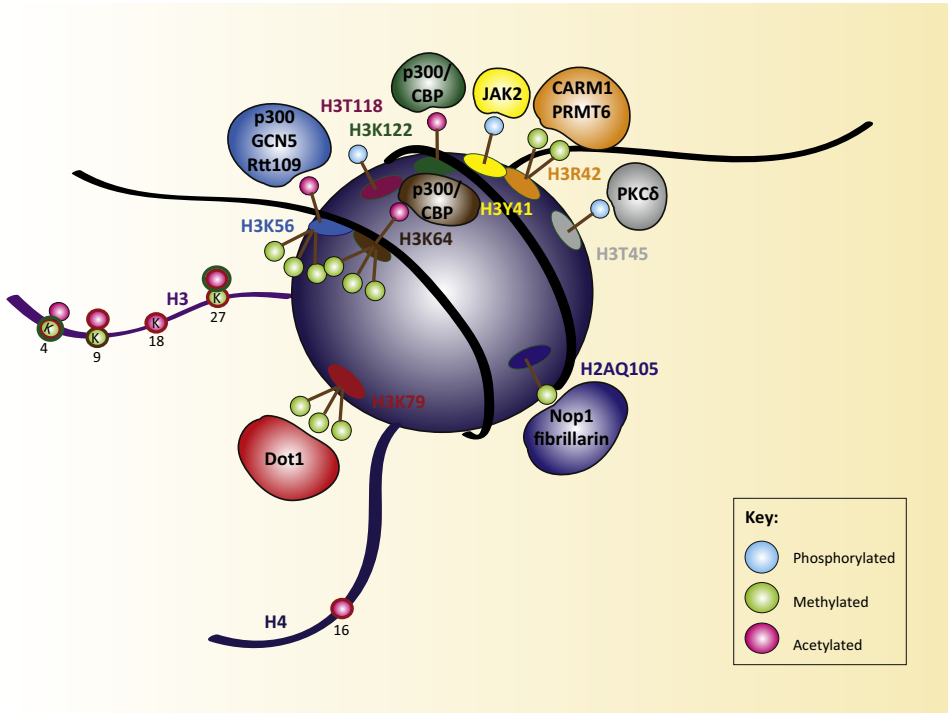
Many modification sites on the lateral surface can have dual roles, depending on the moieties that they acquire. For example, H3K56 can be either methylated or acetylated, with different

consequences [56,57]. H3K56ac is a largely activating modification [58], whereas H3K56me3 is found on repressed genomic regions [56]. Thus, different modifications on the same site can correlate with opposing gene states.

H3K56 is found on the part of the lateral surface of the nucleosome that contacts DNA [59] and is located close to the DNA entry/exit site (Figure 2). H3K56 acetylation was first described in *S. cerevisiae* [60]. H3K56ac is thought to be catalysed by Regulator of Ty1 Transposition 109 (Rtt109) in yeast [61,62] and p300 [63] or GCN5 [64] in human cells (Figure 4). Asf1 is a histone chaperone that cooperates with Rtt109 to promote H3K56 acetylation [65]. Without it, H3K56 acetylation cannot occur [66]. H3K56ac is deposited on newly synthesised H3 [67] and fluctuates in a cell cycle-dependent manner, reaching low levels in G2 [59,68].

H3K56ac also has a role in the activation of transcription (Figure 3) [57]. It increases breathing of the DNA by loosening its grip on the entry and exit sites of the nucleosome [68,69]. H3K56ac is enriched on active genes [58]. This is similar to the role of H3K122ac, which decreases nucleosome binding and thus increases transcription. H3K56ac is deposited on active genes in many systems of gene activation, including *Notch* [70] and Ras/Phosphoinositide-3-kinase (PI3K) [71].

Interestingly, H3K56ac also affects epigenetic marks in the vicinity. The inhibition of Sir6, which deacetylates H3K56, increases 5-hydroxymethyl cytosine (5hmC) on some genes [72]. 5hmC is part of the pathway to DNA demethylation [73]. Therefore, the presence of this activating mark



Trends in Genetics

Figure 4. Schematic of a Nucleosome Detailing the Locations of Globular Domain Modifications, the Enzymes Responsible for Their Deposition, and Their Interplay with Histone Tail Modifications. The colours surrounding each histone tail modification show which globular domain modifications they influence and/or correlate with. Abbreviations: CARM1, Coactivator-associated arginine methyltransferase 1; CBP, CREB-binding protein; Dot1, Disruptor of telomeric silencing 1; JAK2, Janus kinase 2; Nop1, Nucleolar protein 1; PKC, protein kinase C;PRMT6, protein arginine methyltransferase 6; Rtt109, Regulator of Ty1 Transposition109.

on the lateral surface may somehow signal to the cell that DNA demethylation needs to take place.

H3K56ac also has an interesting interplay with transcription factors. It binds to OCT4 in human ESCs (hESCs) [74]. The depletion of Anti-Silencing Function 1A Histone Chaperone (ASF1A), the chaperone required for H3K56 acetylation, reduces the expression of a set of pluripotency-associated genes and increases the expression of a panel of differentiation markers [74]. However, H3K56ac is mainly catalysed on free H3, which is subsequently incorporated into nucleosomes. Thus, ASF1A depletion would reduce histone incorporation into chromatin as well as affecting H3K56ac levels directly by its interaction with H3K56 acetyltransferases [75]. Therefore, the effect on pluripotency may be due to altered nucleosome occupancy and/or a direct result of H3K56ac reduction. Thus, H3K56ac might mesh both with chromatin structure and transcription factors to determine active chromatin signatures.

H3K56 acetylation also has many links to the DNA damage response of the cell. It appears to persist during G2 in the case of DNA damage [68], possibly due to the downregulation of its deacetylases [76]. However, other groups have described a reduction in H3K56ac levels in response to DNA damage [64]. In any case, H3K56ac levels could form a flag for the DNA damage response. Recent findings support this hypothesis [77] and show that H3K56 acetylation may also contribute to the pathway of chromatin reassembly after DNA repair has been completed [78]. In conclusion, H3K56ac may have a role in signalling DNA damage and its repair.

However, H3K56 methylation has a different distribution and opposing effects on gene expression compared with H3K56 acetylation. H3K56 can be either monomethylated [79,80] or trimethylated [56]. Monomethylated H3K56 is thought to have a role in cell cycle progression [79]. The trimethylated version of H3K56 appears to have a completely different role and is associated with transcriptional silencing. The histone methyltransferases SUV39H1/2 are thought to be indirectly responsible for its deposition [56].

H3K56me3 is found at DAPI-dense chromocentres in interphase and in heterochromatic foci in mitotic cells [56]. It is also enriched at major satellite repeats, indicating a role in chromatin compaction and an association with repressive chromatin (Figure 3).

In conclusion, H3K56 can be either acetylated or methylated with different consequences for the cell. This dichotomy is not uncommon because a second residue on the lateral surface of the histone can receive two different modifications with different consequences. H3K64 can also be acetylated, which correlates with transcriptional activation, or methylated, which is associated with repressed regions (Figure 3) [25,81].

H3K64 acetylation is one of the most recently described globular domain modifications [25]. H3K64ac is found at the transcriptional start sites of active genes and its enrichment corresponds directly to the mRNA output of the gene. It is also found on active enhancers. During ESC differentiation, H3K64ac is enriched on the promoters of pluripotency-associated genes and its enrichment subsequently shifts to differentiation genes, showing that it reflects the gene expression state of the cells [25]. Furthermore, H3K64ac is most enriched on the H3.3 variant, which is associated with active transcription. p300/CREB-binding protein (CBP) are the main enzymes responsible for H3K64 acetylation (Figure 4) [25], indicating that H3K64ac could co-localise with the many other histone acetyl marks that p300/CBP catalyses.

H3K64ac may affect chromatin structure directly by decreasing nucleosome stability [25]. H3K64ac also increases nucleosome eviction in *in vitro* assays. Both of these effects increase

genes' accessibility to the transcriptional machinery of the cell. In support of this, H3K64 acetylation hastens the transcriptional induction of key TPA-dependent genes in *in vivo* gene induction assays [25].

Similarly to H3K56, H3K64 can also be methylated. This mark does not co-localise with acetylated H3K64 [25,82], indicating that the marks may be found on functionally distinct regions. Similar to the effect seen on H3K56, H3K64 trimethyl marks [80] are found on repressed areas of the genome, such as pericentromeric heterochromatin [81], in contrast to the acetylated residues, which are found on active regions (Figure 3) [25]. In mESCs, H3K64me3 is associated with many repetitive elements, including Intracisternal A Particle (IAPs), LINE-1 (L1), and subtelomeric repeats. By contrast, H3K64ac is not enriched on these regions [25], further underlining the opposing roles of these two marks. Furthermore, when imprinting control regions are analysed by ChIP, H3K64ac is found on the active allele, whereas H3K64me3 localises to the inactive one [25]. They are differently distributed during early embryonic development [82]. Thus, acetylation and methylation can be considered as two opposing facets of the action of H3K64.

The role of H3K64me3 is also interlaced with that of DNA methylation. During embryonic development, H3K64me3 is found in the oocyte [81], where it co-localises with areas of DNA methylation [83]. H3K64me3 is then removed between fertilisation and the two-cell stage [81,82], when imprinting erasure has taken place and the cell has lower levels of DNA methylation. A similar pattern occurs during primordial germ cell (PGC) maturation: H3K64me3 is found in migrating PGCs at E10.5 when they reach the genital ridge, but is lost at E12.5, when imprinting erasure is taking place and chromatin has to be accessible. Then it is slowly regained [81].

Despite its association with DNA methylation, H3K64me3 deposition does not require DNA methylation as a foothold because the deletion of all three DNA methyltransferase (DNMT) enzymes *in vitro* does not reduce H3K64me3 [83]. Therefore, it is unlikely that H3K64me3 is a direct consequence of DNA methylation.

H3K64me3 also interplays with H3K9me3, because they co-localise on many genomic targets [81]. Similarly H3K64me3 levels are decreased by the deletion of Suppressor of variegation 3-9 homolog 1 (*Suv39h1/2*) [81]; however, this effect may be indirect because H3K64me3 may rely on the presence of H3K9me3 for its deposition [83]. Interestingly, *Suv39h1/2*$^{-/-}$ cells still retain H3K64me3 at least at some repetitive elements, indicating that there could be redundant mechanisms for its maintenance at potentially harmful elements in the genome [81].

Novel Chemistry on the Lateral Surface

Most of the modifications described so far occur on lysine residues; however, other residues of the histone core can also be modified. Recently, the modification of arginine residues on the lateral surface was described [84]. Arginines in the globular domain that have been found to be subject to methylation include R52 and 53 of H3, R92 of H4, R77 of H2A, and R76, 83, 89 and 96 of H2B [24]. Many methylated arginines in the histone tail have been studied in detail [85–87]; however, the functional role of many arginine modifications in the histone core region has yet to be addressed. H3R42 is one of the few arginine methylation sites in this area that has been characterised in detail.

H3R42 is localised in the region where the DNA enters and exits the nucleosome, thus the modification of this site could have implications for DNA binding to the nucleosome (Figure 2). The arginine methyltransferases Coactivator-associated arginine methyltransferase 1/Protein arginine N-methyltransferase 4 (CARM1/PRMT4) and PRMT6 carry out H3R42 methylation

(Figure 4) [88]. Both methylate monomethylated R42 more efficiently than unmodified R42 [88]. *In vitro* transcriptional assays indicate that H3R42 methylation increases transcription (Figure 3) [88]. However, many other arginine modifications in the core region remain uncharacterised.

Glutamine residues in the histone globular domain can also be methylated. H2AQ105 was recently identified as a novel methylation site in *S. cerevisiae* by mass spectrometry analysis [89]. The Nucleolar protein 1 (Nop1) complex was identified as being responsible for its catalysis (Figure 4). In human cells, fibrillarin methylates the corresponding glutamine residue, Q105. Unexpectedly, H2AQ104/5me occurs only on rDNA in the nucleolus, and is dependent on transcription by RNA polymerase I (Pol I) in both human and yeast cells. Surprisingly, a Q105A mutation phenocopies H2AQ105 methylation and reduces the binding of the FACT complex to nucleosomes. The FACT complex comprises Spt16/Pob3 and Nhp6a in yeast cells and usually augments transcription by repositioning nucleosomes during transcription by RNA polymerase and facilitating their redeposition after the transcriptional machinery has passed through [90]. Thus, the authors hypothesise that H2AQ105 methylation prevents the binding of the FACT complex to H2A, decreasing nucleosome occupancy on already nucleosome-poor rDNA loci and increasing transcription as a result (Figure 3).

Tyrosine phosphorylation is a fifth type of modification that can occur in the globular domain of the histone and the final novel modification type that we discuss here. There are many phosphorylation sites in the core domain of histones (Figure 2), many of which have only recently been identified. The first of these is H3Y41, which similarly to R42, forms part of the exit site for DNA from the nucleosome. It was recently described to be subject to phosphorylation [91]. Interestingly, this phosphorylation is carried out by Janus kinase 2 (JAK2) (Figure 4), a kinase whose mutation is associated with leukaemia. Thus, cell lines with *JAK2*-activating mutations had elevated H3Y41ph and the activation of JAK2 with interleukin 3 (IL3), platelet-derived growth factor (PDGF), or Leukaemia inhibitory factor (LIF) had the same effect [91].

H3Y41 phosphorylation appears to block the binding of heterochromatin protein 1 $\propto$ (HP1$\propto$) [91]. Therefore, by interfering with the recruitment of a repressor, H3Y41ph may activate genes which are crucial to leukaemic progression (Figure 4). To sum up, similarly to H3K79 methylation, H3Y41ph may leave a disease-associated epigenetic fingerprint, allowing us to see which genes have been activated by oncogenic transformation.

H3T45 is another H3 residue that can be phosphorylated [92]. This mark is catalysed by Protein kinase C δ (PKCδ), downstream of caspase 3 cleavage [92]. Almost all cells that stain positive for H3T45ph are apoptotic. Thus, as well as forming a fingerprint of gene activation, histone marks can also differentiate healthy cells from those undergoing apoptosis (Figure 3).

Other phosphorylation sites in the globular domain of H3 can also affect nucleosome assembly. H3T118 has been described as a novel phosphorylation site. The mutation of T118 to alanine destabilises the nucleosome and increases its repositioning [93], while its mutation to isoleucine increases nucleosome accessibility [94]. Therefore, H3T118 forms a key interface between the nucleosome and the DNA and H3T118 modifications could have significant impacts on chromatin architecture. In agreement with this, the phosphorylation of H3T118 results in enhanced nucleosome mobility, increased nucleosome accessibility, and elevated nucleosome disassembly during SwItch/Sucrose nonfermentable (SWI/SNF)-mediated remodelling [95]. In nucleosome assembly assays using malaria Nap, H3T118ph was described to increase nucleosomal binding to histone chaperones [96]. Therefore, H3T118ph is a modification in the globular domain of histone H3 that can have direct effects on nucleosome assembly and remodelling (Figure 3).

While H3T118ph increases the binding of H3 to Nap, H3K122 acetylation decreases this binding [96]. This indicates that core modifications may oppose each other to regulate chaperone binding and underlines the fact that while the crosstalk between histone tail modifications has been well studied [13], the crosstalk between histone core modifications remains largely unaddressed.

Lateral Influences: The Crosstalk between Histone Tail Modifications and the Lateral Surface

Histone tail modifications are known to influence the deposition of other tail modifications [13]. However, histone tail modifications can also interplay with modifications of the lateral surface (Figure 4). H3K79me3 is found on genomic regions that are also enriched in H3K4me3 [31], indicating that both marks co-localise on active chromatin. Similarly, H3K79me2-enriched regions also have increased H3K4me3 [33,36]. However, it is unclear which mark is deposited first. Furthermore, H3K79 methylation depends on the deposition of H2BK123Ub [34,38]. H3K79me2 also has a reciprocal relationship with some modifications, for example H4K16ac. The mutation of H4K16 to mimic permanent acetylation reduces H3K79me2 levels, whereas the removal of H3K79me2 by Dot1 mutation increases the levels of H4 acetylation [34]. Therefore, H3K79me2/3 marks co-localise with some marks and anticorrelate with others.

As well as being associated with the active chromatin state, H3K79 modifications are also associated with the poised chromatin state. In ESCs, H3K79me1 is enriched at regions where H3K4me3 and H3K27me3 are also found [36,40]. Similarly to H3K79me3, H3K122ac co-localises with not only H3K4me3, but also H2A.Z, acetylated H2A.Z, and H3.3, which are associated with actively transcribed regions. In addition to transcription start sites, H3K122ac is also found on active enhancers along with H3K4me1 and H3K27ac [23].

Repressive lateral surface modifications can also interplay with histone tail modifications. For example, H3K64me3 co-localises with H3K9me3 on many genomic regions [81] and the deletion of *Suv39h1/2*, the enzymes that catalyse H3K9me3, also reduces H3K64me3 levels [81]. H3K64me3 relies on H3K9me3 for its deposition [83]. However, some repetitive elements maintain their H3K64me3 status in *Suv39h1/2*$^{-/-}$ cells, indicating that H3K64me3 is not entirely dependent on H3K9me3 for its maintenance.

Concluding Remarks

Similar to modifications in the tail regions of histones, the modification of histone globular domains can have diverse consequences for the cell. This is particularly evident for residues on the lateral surface of the nucleosome, which directly contact the DNA. Several important themes have emerged in this review. The first is that modifications on the lateral surface of the nucleosome can have direct impacts on transcription despite the fact that, for most of these sites, no specific binders or readers have been identified. For example, H3K122ac can directly increase transcription levels *in vitro* by evicting histones from the DNA. H3K64ac and H3R42ac had stimulatory effects during nucleosome eviction assays and *in vitro* transcription assays, respectively (Figure 3). So far, this appears to be a direct impact of the histone modifications rather than via the recruitment of binding partners. Therefore, histone modifications can be drivers of transcriptional change with causative functions, rather than mere by-products of transcription or markers of chromatin state. However, it is not always easy to uncouple direct and indirect effects. More work is needed to disentangle the *in vivo* direct effects of histone modifications on transcription from alterations in histone modifications that occur downstream of transcriptional change and are simply deposited on already active or inactive regions. Furthermore, the 'readers' of many core histone modifications remain to be identified, leaving the downstream pathways they could initiate largely uncharted (see Outstanding Questions).

Outstanding Questions

To what extent can histone modifications instruct transcriptional change?

When two or more opposing modifications can be placed on the same residue with differing effects, what factors decide which mark is deposited?

How do lateral surface modifications interplay with both histone tail modifications and other lateral surface marks?

How is cell state change influenced by alterations in histone modifications?

What proteins 'read' lateral surface modifications; could these instruct transcriptional alteration?

The second theme that has emerged during this review is the fact that different histone lateral surface modifications on the same residue can have different associations with gene state. H3K56 and H3K64 are two residues on the lateral surface of the nucleosome that illustrate this point, because both can be subjected to acetylation, a mark associated with active chromatin states. Conversely, they can both be methylated and these marks are found on repressed regions. Therefore, by modifying a single residue in the lateral surface region of the nucleosome, the cell can either repress or activate a particular genomic region with differing effects on gene expression (Figure 3).

In conclusion, recent advances in the field have begun to characterise novel modifications in the core domains of histones. There are many modifications that have yet to be characterised. For example, work is currently underway on a whole set of novel histone acylations, including butyrylation and propionylation [97] and crotonylation [84], and insights into these modifications will further influence our knowledge of the roles of histone modifications in chromatin compaction and transcription [14]. The histone globular domain modifications that have already been studied in detail have enabled insights into leukaemia, solid cancers, DNA repair, cellular reprogramming, differentiation, and pluripotency. Therefore, the lateral surface of the nucleosome represents a central area of ongoing research in epigenetics and provides us with key insights into the interface between chromatin regulation and transcriptional control.

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References

1. Bloom, K. and Joglekar, A. (2010) Towards building a chromosome segregation machine. *Nature* 463, 446–456
2. Hadlaczky, G. *et al.* (1986) Direct evidence for the non-random localization of mammalian chromosomes in the interphase nucleus. *Exp. Cell Res.* 167, 1–15
3. Schardin, M. *et al.* (1985) Specific staining of human chromosomes in Chinese hamster x man hybrid cell lines demonstrates interphase chromosome territories. *Hum. Genet.* 71, 281–287
4. Oudet, P. *et al.* (1975) Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* 4, 281–300
5. Luger, K. *et al.* (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389, 251–260
6. Bentley, G.A. *et al.* (1984) Crystal structure of the nucleosome core particle at 16 A resolution. *J. Mol. Biol.* 176, 55–75
7. Kornberg, R.D. and Thomas, J.O. (1974) Chromatin structure; oligomers of the histones. *Science* 184, 865–868
8. Allan, J. *et al.* (1980) The structure of histone H1 and its location in chromatin. *Nature* 288, 675–679
9. Kornberg, R.D. (1974) Chromatin structure: a repeating unit of histones and DNA. *Science* 184, 868–871
10. Olins, A.L. and Olins, D.E. (1974) Spheroid chromatin units (v bodies). *Science* 183, 330–332
11. Wasylyk, B. and Chambon, P. (1979) Transcription by eukaryotic RNA polymerases A and B of chromatin assembled in vitro. *Eur. J. Biochem.* 98, 317–327
12. Lee, D.Y. *et al.* (1993) A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* 72, 73–84
13. Kouzarides, T. (2007) Chromatin modifications and their function. *Cell* 128, 693–705
14. Kebede, A.F. *et al.* (2015) Novel types and sites of histone modifications emerge as players in the transcriptional regulation contest. *FEBS J.* 282, 1658–1674
15. Shogren-Knaak, M. *et al.* (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844–847
16. Akhtar, A. and Becker, P.B. (2000) Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol. Cell* 5, 367–375
17. Lu, X. *et al.* (2008) The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. *Nat. Struct. Mol. Biol.* 15, 1122–1124
18. Wysocka, J. *et al.* (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442, 86–90
19. Margueron, R. *et al.* (2005) The key to development: interpreting the histone code? *Curr. Opin. Genet. Dev.* 15, 163–176
20. Vettese-Dadey, M. *et al.* (1996) Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. *EMBO J.* 15, 2508–2518
21. Clements, A. *et al.* (2003) Structural basis for histone and phosphohistone binding by the GCN5 histone acetyltransferase. *Mol. Cell* 12, 461–473
22. Ausio, J. *et al.* (1989) Use of selectively trypsinized nucleosome core particles to analyze the role of the histone 'tails' in the stabilization of the nucleosome. *J. Mol. Biol.* 206, 451–463
23. Tropberger, P. *et al.* (2013) Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. *Cell* 152, 859–872
24. Cosgrove, M.S. *et al.* (2004) Regulated nucleosome mobility and the histone code. *Nat. Struct. Mol. Biol.* 11, 1037–1043
25. Di Cerbo, V. *et al.* (2014) Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. *Elife* 3, e01632
26. Zhang, K. *et al.* (2002) Identification of acetylation and methylation sites of histone H3 from chicken erythrocytes by high-accuracy matrix-assisted laser desorption ionization-time-of-flight, matrix-assisted laser desorption ionization-postsource decay, and nanoelectrospray ionization tandem mass spectrometry. *Anal. Biochem.* 306, 259–269
27. van Leeuwen, F. *et al.* (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 109, 745–756

28. Feng, Q. *et al.* (2002) Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr. Biol.* 12, 1052–1058
29. Ng, H.H. *et al.* (2002) Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* 16, 1518–1527
30. Jones, B. *et al.* (2008) The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. *PLoS Genet.* 4, e1000190
31. Nakanishi, S. *et al.* (2009) Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. *J. Cell Biol.* 186, 371–377
32. Barry, E.R. *et al.* (2009) ES cell cycle progression and differentiation require the action of the histone methyltransferase Dot1L. *Stem Cells* 27, 1538–1547
33. Guenther, M.G. *et al.* (2007) A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 130, 77–88
34. Ng, H.H. *et al.* (2003) Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1820–1825
35. Schubeler, D. *et al.* (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* 18, 1263–1271
36. Steger, D.J. *et al.* (2008) DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol. Cell. Biol.* 28, 2825–2839
37. Fingerman, I.M. *et al.* (2007) A charge-based interaction between histone H4 and Dot1 is required for H3K79 methylation and telomere silencing: identification of a new trans-histone pathway. *Genes Dev.* 21, 2018–2029
38. Briggs, S.D. *et al.* (2002) Gene silencing: trans-histone regulatory pathway in chromatin. *Nature* 418, 498
39. Vakoc, C.R. *et al.* (2006) Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol. Cell. Biol.* 26, 9185–9195
40. Bernstein, B.E. *et al.* (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125, 315–326
41. Okada, Y. *et al.* (2005) hDOT1L links histone methylation to leukemogenesis. *Cell* 121, 167–178
42. Okada, Y. *et al.* (2006) Leukaemic transformation by CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. *Nat. Cell Biol.* 8, 1017–1024
43. Singh, P. *et al.* (2010) Allele-specific H3K79 Di- versus trimethylation distinguishes opposite parental alleles at imprinted regions. *Mol. Cell Biol.* 30, 2693–2707
44. Ooga, M. *et al.* (2008) Changes in H3K79 methylation during preimplantation development in mice. *Biol. Reprod.* 78, 413–424
45. Onder, T.T. *et al.* (2012) Chromatin-modifying enzymes as modulators of reprogramming. *Nature* 483, 598–602
46. Zhang, L. *et al.* (2014) Inhibition of histone H3K79 methylation selectively inhibits proliferation, self-renewal and metastatic potential of breast cancer. *Oncotarget* 5, 10665–10677
47. Krivtsov, A.V. *et al.* (2008) H3K79 methylation profiles define murine and human MLL-AF4 leukemias. *Cancer Cell* 14, 355–368
48. Kuntimaddi, A. *et al.* (2015) Degree of recruitment of DOT1L to MLL-AF9 defines level of H3K79 Di- and tri-methylation on target genes and transformation potential. *Cell Rep.* 11, 808–820
49. Li, Y. *et al.* (2014) AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation. *Cell* 159, 558–571
50. Wysocki, R. *et al.* (2005) Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. *Mol. Cell. Biol.* 25, 8430–8443
51. Giannattasio, M. *et al.* (2005) The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. *J. Biol. Chem.* 280, 9879–9886
52. Manohar, M. *et al.* (2009) Acetylation of histone H3 at the nucleosome dyad alters DNA-histone binding. *J. Biol. Chem.* 284, 23312–23321
53. Cocklin, R.R. and Wang, M. (2003) Identification of methylation and acetylation sites on mouse histone H3 using matrix-assisted laser desorption/ionization time-of-flight and nanoelectrospray ionization tandem mass spectrometry. *J. Protein Chem.* 22, 327–334
54. Hall, M.A. *et al.* (2009) High-resolution dynamic mapping of histone-DNA interactions in a nucleosome. *Nat. Struct. Mol. Biol.* 16, 124–129
55. Tropberger, P. and Schneider, R. (2013) Scratching the (lateral) surface of chromatin regulation by histone modifications. *Nat. Struct. Mol. Biol.* 20, 657–661
56. Jack, A.P. *et al.* (2013) H3K56me3 is a novel, conserved heterochromatic mark that largely but not completely overlaps with H3K9me3 in both regulation and localization. *PLoS ONE* 8, e51765
57. Williams, S.K. *et al.* (2008) Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 9000–9005
58. Xu, F. *et al.* (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell* 121, 375–385
59. Ozdemir, A. *et al.* (2005) Characterization of lysine 56 of histone H3 as an acetylation site in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280, 25949–25952
60. Hyland, E.M. *et al.* (2005) Insights into the role of histone H3 and histone H4 core modifiable residues in Saccharomyces cerevisiae. *Mol. Cell. Biol.* 25, 10060–10070
61. Schneider, J. *et al.* (2006) Rtt109 is required for proper H3K56 acetylation: a chromatin mark associated with the elongating RNA polymerase II. *J. Biol. Chem.* 281, 37270–37274
62. Han, J. *et al.* (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* 315, 653–655
63. Das, C. *et al.* (2009) CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* 459, 113–117
64. Tjeertes, J.V. *et al.* (2009) Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. *EMBO J.* 28, 1878–1889
65. Driscoll, R. *et al.* (2007) Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* 315, 649–652
66. Recht, J. *et al.* (2006) Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6988–6993
67. Li, Q. *et al.* (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* 134, 244–255
68. Masumoto, H. *et al.* (2005) A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* 436, 294–298
69. Neumann, H. *et al.* (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. *Mol. Cell* 36, 153–163
70. Skalska, L. *et al.* (2015) Chromatin signatures at Notch-regulated enhancers reveal large-scale changes in H3K56ac upon activation. *EMBO J.* 34, 1889–1904
71. Liu, Y. *et al.* (2012) Oncogene Ras/phosphatidylinositol 3-kinase signaling targets histone H3 acetylation at lysine 56. *J. Biol. Chem.* 287, 41469–41480
72. Etchegaray, J.P. *et al.* (2015) The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine. *Nat. Cell Biol.* 17, 545–557
73. Tahiliani, M. *et al.* (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324, 930–935
74. Tan, Y. *et al.* (2013) Acetylated histone H3K56 interacts with Oct4 to promote mouse embryonic stem cell pluripotency. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11493–11498
75. Klopf, E. *et al.* (2009) Cooperation between the INO80 complex and histone chaperones determines adaptation of stress gene transcription in the yeast Saccharomyces cerevisiae. *Mol. Cell. Biol.* 29, 4994–5007
76. Maas, N.L. *et al.* (2006) Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. *Mol. Cell* 23, 109–119

77. Zhu, Q. *et al.* (2015) Damaged DNA-binding protein down-regulates epigenetic mark H3K56Ac through histone deacetylase 1 and 2. *Mutat. Res.* 776, 16–23
78. Chen, C.C. *et al.* (2008) Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell* 134, 231–243
79. Yu, Y. *et al.* (2012) Histone H3 lysine 56 methylation regulates DNA replication through its interaction with PCNA. *Mol. Cell* 46, 7–17
80. Peters, A.H. *et al.* (2003) Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* 12, 1577–1589
81. Daujat, S. *et al.* (2009) H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming. *Nat. Struct. Mol. Biol.* 16, 777–781
82. Ziegler-Birling, C. *et al.* (2015) Dynamics of histone H3 acetylation in the nucleosome core during mouse pre-implantation development. *Epigenetics* Published online October 19, 2015. http://dx.doi.org/10.1080/15592294.2015.1103424
83. Lange, U.C. *et al.* (2013) Dissecting the role of H3K64me3 in mouse pericentromeric heterochromatin. *Nat. Commun.* 4, 2233
84. Tan, M. *et al.* (2011) Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* 146, 1016–1028
85. An, W. *et al.* (2004) Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. *Cell* 117, 735–748
86. Ying, Z. *et al.* (2015) Histone arginine methylation by PRMT7 controls germinal center formation via regulating Bcl6 transcription. *J. Immunol.* 195, 1538–1547
87. Wang, H. *et al.* (2001) Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* 293, 853–857
88. Casadio, F. *et al.* (2013) H3R42me2a is a histone modification with positive transcriptional effects. *Proc. Natl. Acad. Sci. U.S.A.* 110, 14894–14899
89. Tessarz, P. *et al.* (2014) Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature* 505, 564–568
90. Belotserkovskaya, R. *et al.* (2003) FACT facilitates transcription-dependent nucleosome alteration. *Science* 301, 1090–1093
91. Dawson, M.A. *et al.* (2009) JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature* 461, 819–822
92. Hurd, P.J. *et al.* (2009) Phosphorylation of histone H3 Thr-45 is linked to apoptosis. *J. Biol. Chem.* 284, 16575–16583
93. Muthurajan, U.M. *et al.* (2004) Crystal structures of histone Sin mutant nucleosomes reveal altered protein–DNA interactions. *EMBO J.* 23, 260–271
94. Kurumizaka, H. and Wolffe, A.P. (1997) Sin mutations of histone H3: influence on nucleosome core structure and function. *Mol. Cell. Biol.* 17, 6953–6969
95. North, J.A. *et al.* (2011) Phosphorylation of histone H3(T118) alters nucleosome dynamics and remodeling. *Nucleic Acids Res.* 39, 6465–6474
96. Kumar, A. *et al.* (2012) Structural delineation of histone post-translation modifications in histone-nucleosome assembly protein complex. *J. Struct. Biol.* 180, 1–9
97. Chen, Y. *et al.* (2007) Lysine propionylation and butyrylation are novel post-translational modifications in histones. *Mol. Cell Proteomics* 6, 812–819
98. Barber, C.M. *et al.* (2004) The enhancement of histone H4 and H2A serine 1 phosphorylation during mitosis and S-phase is evolutionarily conserved. *Chromosoma* 112, 360–371
99. Fusauchi, Y. and Iwai, K. (1984) Tetrahymena histone H2A. Acetylation in the N-terminal sequence and phosphorylation in the C-terminal sequence. *J. Biochem.* 95, 147–154
100. Suka, N. *et al.* (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. *Mol. Cell* 8, 473–479
101. Baarends, W.M. *et al.* (2007) Increased phosphorylation and dimethylation of XY body histones in the Hr6b-knockout mouse is associated with derepression of the X chromosome. *J. Cell Sci.* 120, 1841–1851
102. Wang, H. *et al.* (2004) Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 431, 873–878
103. Cheung, W.L. *et al.* (2003) Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* 113, 507–517
104. Maile, T. *et al.* (2004) TAF1 activates transcription by phosphorylation of serine 33 in histone H2B. *Science* 304, 1010–1014
105. Golebiowski, F. and Kasprzak, K.S. (2005) Inhibition of core histones acetylation by carcinogenic nickel(II). *Mol. Cell. Biochem.* 279, 133–139
106. Robzyk, K. *et al.* (2000) Rad6-dependent ubiquitination of histone H2B in yeast. *Science* 287, 501–504
107. Santos-Rosa, H. *et al.* (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* 419, 407–411
108. Roguev, A. *et al.* (2001) The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. *EMBO J.* 20, 7137–7148
109. Strahl, B.D. *et al.* (1999) Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in Tetrahymena. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14967–14972
110. Rea, S. *et al.* (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593–599
111. Chen, D. *et al.* (1999) Regulation of transcription by a protein methyltransferase. *Science* 284, 2174–2177
112. Bauer, U.M. *et al.* (2002) Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Rep.* 3, 39–44
113. Nishioka, K. *et al.* (2002) PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol. Cell* 9, 1201–1213
114. Schotta, G. *et al.* (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev.* 18, 1251–1262
115. Zhang, K. *et al.* (2002) Histone acetylation and deacetylation: identification of acetylation and methylation sites of HeLa histone H4 by mass spectrometry. *Mol. Cell. Proteomics* 1, 500–508
116. Zhang, H. *et al.* (2013) PP1alpha, PP1beta and Wip-1 regulate H4S47 phosphorylation and deposition of histone H3 variant H3.3. *Nucleic Acids Res.* 41, 8085–8093
117. Yang, X. *et al.* (2011) HAT4, a Golgi apparatus-anchored B-type histone acetyltransferase, acetylates free histone H4 and facilitates chromatin assembly. *Mol. Cell* 44, 39–50
118. Martinato, F. *et al.* (2008) Analysis of Myc-induced histone modifications on target chromatin. *PLoS ONE* 3, e3650