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# Histone and chromatin cross-talk

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Chromatin is the physiologically relevant substrate for all genetic processes inside the nuclei of eukaryotic cells. Dynamic changes in the local and global organization of chromatin are emerging as key regulators of genomic function. Indeed, a multitude of signals from outside and inside the cell converges on this gigantic signaling platform. Numerous post-translational modifications of histones, the main protein components of chromatin, have been documented and analyzed in detail. These 'marks' appear to crucially mediate the functional activity of the genome in response to upstream signaling pathways. Different layers of cross-talk between several components of this complex regulatory system are emerging, and these epigenetic circuits are the focus of this review.

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**Abbreviations**

| | |
|---|---|
| **bromodomain** | brm (brahma)-like domain |
| **ChIP** | chromatin immunoprecipitation |
| **chromo** | chromatin-organization modifier |
| **HAT** | histone acetyltransferase |
| **HDAC** | histone deacetylase |
| **HMT** | histone methyltransferase |
| **HP1** | heterochromatin protein 1 |
| **IES** | internal eliminated sequences |
| **RNAi** | RNA interference |
| **shRNA** | small heterochromatic RNA |
| **siRNA** | small interfering RNA |

## Introduction

Within the eukaryotic cell nucleus, genetic information is organized in a highly conserved structural polymer, termed chromatin, which supports and controls the crucial functions of the genome. The chromatin template undergoes dynamic changes during many genetic processes. These include necessary structural reorganizations that occur during DNA replication and cell cycle progression, spatially and temporally coordinated gene expression, as well as DNA repair and recombination events. The fundamental repeating unit of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around an octamer of core histone proteins — H2A, H2B, H3, and H4. Linker histones of the H1 class associate with DNA between single nucleosomes establishing a higher level of organization, the so-called 'solenoid' helical fibers (30 nm fibers). Chromatin architecture beyond the 30 nm fibers is less clear, but folding and unfolding of putative superstructures are thought to have a pronounced impact on genomic function and gene activity.

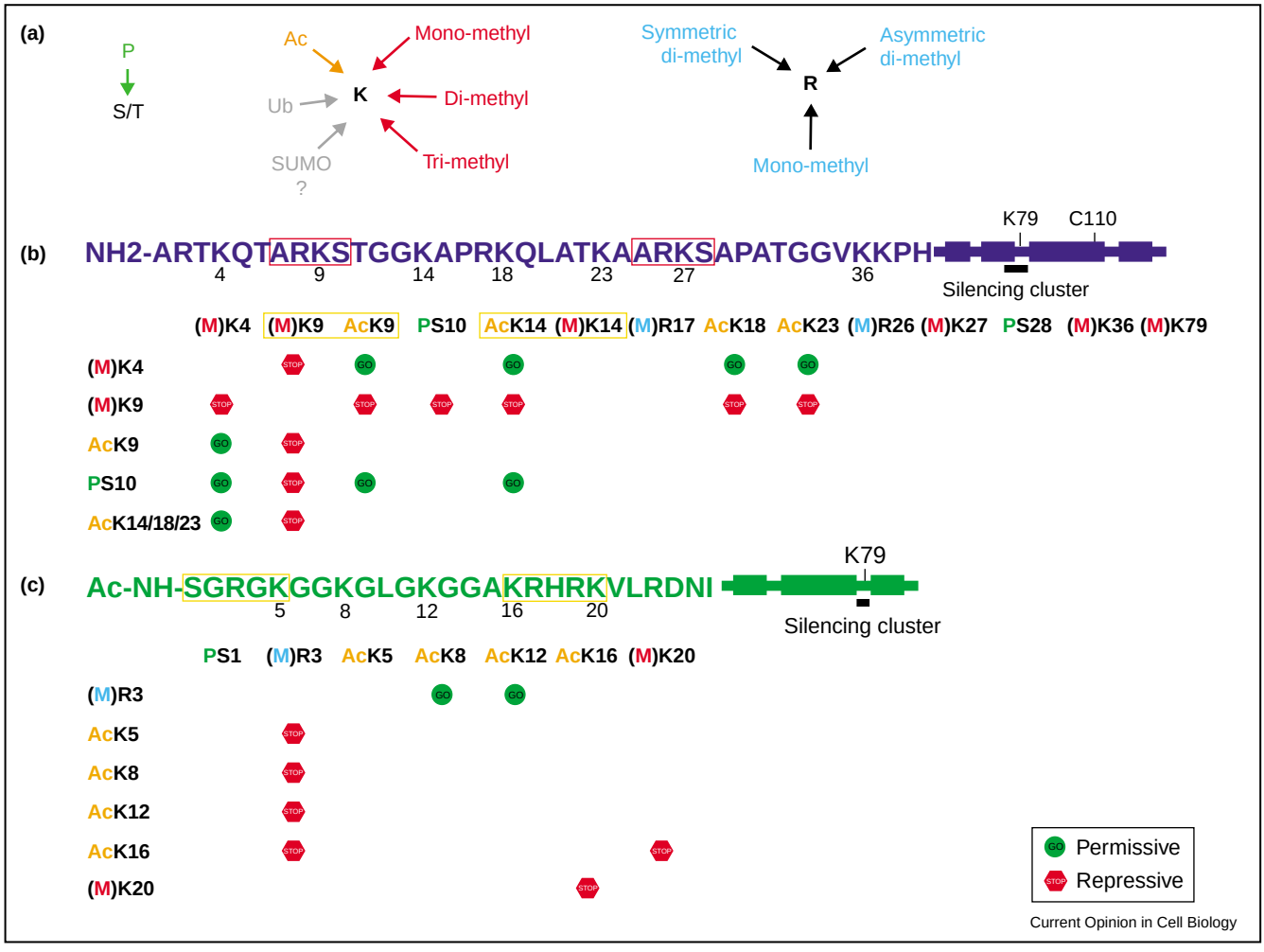
Core histone proteins are evolutionarily conserved and consist mainly of flexible amino-terminal tails protruding outward from the nucleosome, and globular carboxy-terminal domains making up the nucleosome scaffold. Histones function as acceptors for a variety of post-translational modifications, including acetylation, methylation and ubiquitination of lysine (K) residues, phosphorylation of serine (S) and threonine (T) residues, and methylation of arginine (R) residues (Figure 1a). The different histone modifications and the corresponding enzymatic systems that maintain them have been reviewed extensively in the recent literature (e.g. [1–5]). Combinations of post-translational marks on single histones, single nucleosomes and nucleosomal domains establish local and global patterns of chromatin modification that may specify unique downstream functions ([6,7]). These patterns can be altered by multiple extracellular and intracellular stimuli, and chromatin itself has been proposed to serve as signaling platform and to function as a genomic integrator of various signaling pathways [8].

In many cellular regulatory networks, distinct binding modules help to integrate different branches of signal input and several signaling transduction pathways converge on central platforms. Indeed, cross-talk between different signaling cascades has emerged as a paradigm of cell biology [9,10]. Here, we expand this concept to histones and focus on cross-talk mechanisms and signaling systems that direct the local and global functions of chromatin. We explore the 'communication' between different post-translational modifications of histones and the 'interaction' of histone modifications with other chromatin components on multiple structural and functional levels.

## Cross-talk between histone modifications

One major challenge in chromatin biology is connecting particular modifications with distinct biological functions and vice versa. One of the better-understood histone modifications in that aspect is histone acetylation. It is

**Figure 1**




Cross-talk at the level of a single histone tail. **(a)** Potential 'choices' of the modification status of different histone residues. P, phosphorylation of serine or threonine (S/T); Ac, acetylation of lysine (K), mono-, di- and tri-methylation of lysine, or mono- or di-methylation (symmetric or asymmetric) of arginine (R); Ub, mono-ubiquitination of lysine; SUMO, sumolation of lysine (note: sumolation has not yet been detected on cellular histones). **(b)** Local cross-talk on the human H3 amino-terminal tail domain. The sequence of the amino-terminal tail of H3 (amino acids 1–40) and the four α-helices (represented by boxes) of the globular domain of H3 are shown (human H3; note the position of the highly conserved cysteine 110, C110). Sites of known modifications are listed (M, mono-, di- or tri-methylation). K9 and K14 have been found to be methylated or acetylated (yellow box). ARKS repeats that contain two sites of methylation (K9 and K27), as well as known sites of phosphorylation (S10 and S28), are highlighted. Primary modifications that positively (green, 'go' or permissive) or negatively (red, 'stop' or repressive) influence the modification of other sites in *in vitro* enzymatic assays are listed on the left. The situation is likely to be more complicated *in vivo*, and enzymes that modify the same site might be influenced differently by the modification-state of their substrate. For example, although methylation on K4 impairs the ability of Su(var)3-9 to methylate K9, and methylation on K9 inhibits the enzymatic activity of SET7/9 to methylate K4 [26], the *Drosophila* HMT, Ash1, seems to be able to methylate both K4 and K9 at the same time [59]. **(c)** Local cross-talk on the human H4 amino-terminal tail domain. The sequence of the amino-terminal tail of H4 (amino acids 1–26) and the three α-helices (represented by boxes) of the globular domain of H4 are shown (human sequence). Interference and 'communication' between known modifications are outlined as in (b). The extreme amino-terminal residues, SGRGK (boxed), are known to be modified by phosphorylation (S1), methylation (R3), and acetylation (K5) in some species. As such, these residues might form a 'modification cassette' that remains poorly understood. The patch of basic residues (KRHRK) can be acetylated (K16) or methylated (K20), which represents a mutually exclusive pair of modifications that either facilitate or repress gene expression [31]. For both H3 (b) and H4 (c), short sequence patches in the globular domains that were shown to play an important role in gene silencing in budding yeast are underlined [41••].

now generally accepted that hyperacetylated histones are mostly associated with activated genomic regions, at both local and global levels. By contrast, deacetylation (leading to hypoacetylation) mainly results in repression and silencing [7,11].

Interestingly, histone methylation appears to have multiple effects on chromatin function in a system- and site-specific manner. Methylation of H3 on K9, for example, is largely associated with silencing and repression in many species. Methylation of H3 on K4, on the other hand, is

most often associated with active or permissive chromatin regions. However, deletion of the H3-K4 HMT (histone methyltransferase), Set1, in budding yeast causes defects in rDNA silencing [12,13]. These findings raise the question of whether methylation of H3 on K4 is also involved in gene repression in this organism. Similarly, methylation of H3-K36 has been suggested to be involved in transcriptional repression [14], but the corresponding modifying enzyme, Set2, has been found in complex with actively transcribing (or elongation engaged) RNA pol II [15]. Along with the dual personality of the phosphorylation of H3 at S10, which has been implicated in not only transcriptional activation but also mitotic chromosome condensation [16], these results argue that single histone modifications may have distinct biological effects depending on their context.

The findings that a particular post-translational modification might mediate separate, and sometimes opposing, physiological processes led to the suggestion that multiple readouts of a certain covalent mark could be obtained by various combinations of different modifications in the same chromatin region [6,17]. Indeed, the use of antibodies that recognize such combinations of post-translational marks, and the more recent application of novel mass spectrometry approaches, have verified that particular sets of modifications might occur concomitantly on the same histone tail [16,18,19]. Although the field is far away from deciphering the specific modification patterns at the level of single histones, single nucleosomes, and nucleosomal domains, mounting evidence suggests that different histone modifications can influence or 'communicate' with each other on several levels.

## Cross-talk choices

An ever growing number of modification sites on both histone-tail and -non-tail domains have been identified (for reference, see Figure 1 and [1]). Whereas serine and threonine residues are well-known phospho-acceptor sites, lysine and arginine residues have multiple choices of post-translational modification possibilities (Figure 1a). For example, lysine residues in histones can be modified by acetylation, mono-ubiquitination or mono-, di-, and tri-methylation. Similarly, arginines might be mono- or di-methylated (symmetric or asymmetric) [1,20]. Although it remains unclear as to what extent, if at all, individual residues undergo 'choices' of modification, it is well documented that H3-K9 and H3-K14 can be either acetylated or (mono-, di-, tri-) methylated [18,19]. Obviously, different marks on the same site cannot co-exist, and therefore, they exclude each other. An acetyl group, for example, must be removed before a methyl group can be added and complexes that contain both histone deacetylases (HDACs) and HMTs have now been identified [21–23]. Genetic studies in *Schizosaccharomyces pombe* have further shown that the HDAC, Clr6, is necessary for methylation at H3-K9 by the Clr4 HMT to occur [24].

It seems obvious that different modifications of a particular site can have different readouts and biological functions. Nevertheless, we now also know that the exact state of methylation (i.e. mono-, di- or tri-methylation) of a single lysine residue has an impact on physiological processes. For example, it was recently shown that di-methylation of H3-K4 occurs at both inactive and active euchromatic genes, whereas tri-methylation is present exclusively at active genes [25••]. Similar studies investigating other sites of methylation are underway, and it will be interesting to see what additional layers of complexity will be added to histone modifications by the modification choice of a single residue.

## Cross-talk at the level of a single histone tail

Many of the enzymes that post-translationally modify histones display a high degree of specificity not only towards a particular site, but also towards the pre-existing modification-state of their substrate. So far the amino-terminal tail of H3 has the highest density of post-translational modifications mapped among all histones, and a complex pattern of putative combinations of marks is emerging (Figure 1b). Methylation on H3-K9, for example, appears to trigger sequential events leading ultimately to transcriptional repression [26]. At least *in vitro*, this mark can inhibit acetylation of the H3 tail (on K14, K18 and K23) by histone acetyltransferases (HATs) (e.g. p300) [26], and methylation of H3 on K4 by HMTs (e.g. Set7) [26]. By contrast, H3-K4 methylation inhibits K9 methylation by Su(var)3-9, but promotes acetylation of H3 by p300 [26].

Remarkably, the choice of methylation of H3 on K9 could be dictated by H3-S10 phosphorylation. In mammalian cells, this mark not only inhibits methylation on K9 [27], but also precedes and promotes acetylation on K14 following specific signals ([16]; see also [8] and references therein). In *Saccharomyces cerevisiae*, Snf1 and Gcn5 — the enzymes that phosphorylate H3-S10 and acetylate H3-K14, respectively — appear to work synergistically to mediate gene activation [28]. Moreover, acetylation on H3-K9 and H3-K14 stimulates methylation of H3-K4 by the HMT, MLL (mixed lineage leukemia protein) [29]. This result is consistent with the enrichment of histones carrying these modifications on *HOX* gene promoters as shown by chromatin immunoprecipitation (ChIP) assays [29]. Conversely, methylation on H3-K4 itself can stimulate the subsequent acetylation of H3 (as discussed above). *In vitro*, further interplay is seen at the level of H3-S10 phosphorylation by the mitotic kinase Ipl1/aurora, which is stimulated when H3-K9 and H3-K14 are acetylated [27].

Additional tail-restricted cross-talk is emerging from studies on modifications of H4 (Figure 1c). Methylation of H4-R3 by PRMTI, for example, is heavily impaired by acetylation of H4 on K5, K8, K12, and K16 [30]. By

contrast, acetylation of H4 on K8 and K12 by the HAT p300 is elevated after methylation of R3 [30]. Also, it has been suggested that methylation of K20 and acetylation of K16 are mutually exclusive [31]. The local cross-talk situation is likely to be more complicated *in vivo*, and enzymes that modify the same site might be influenced differently by the modification-state of their substrate.

### Cross-talk at the level of nucleosomes and nucleosomal domains

Perhaps more fascinating than the direct synergism/antagonism or 'communication' of adjacent modifications in the same histone tail ('*cis*' effects) is the unexpected discovery that modifications on different histones can affect each other ('*trans*' effects) [26,32••,33•–35•]. These effects might be restricted to a single nucleosome or might affect larger nucleosomal arrays or domains (Figure 2). For example, *in vitro* studies using p300 showed that this HAT acetylates both H3 and H4 especially in nucleosomes where H3 is methylated on K4 [26]. By contrast, methylation of H3 on K9 significantly inhibits the activity of p300 towards nucleosomal histones, H3 as well as H4 [26].

Another intriguing '*trans*' cross-talk originates from work in *S. cerevisiae* linking ubiquitination of H2B to methylation of H3 (Figure 2a) [32••,33•–35•]. Ubiquitination of H2A and H2B in mammalian cells had been known for a long time (e.g. ubiquitin was discovered on H2A [36]), but without an obvious link to protein turnover, the consequences and functions of histone mono-ubiquitination had been elusive. With the discovery of mono-ubiquitination of H2B in yeast, genetic studies of histone ubiquitination became possible [37]. Surprisingly, mutagenesis of either the ubiquitin acceptor site, H2B-K123 (the equivalent of human H2B-K120), or disruption of the ubiquitin-conjugating enzyme Rad6/Ubc2 in this organism results in a striking loss of methylation at H3-K4 and H3-K79 [32••,33•–35•]. Altogether, these results indicate that ubiquitination of H2B is a prerequisite for methylation of H3 on K4 and K79. On the other hand, abolishment of H3-K4 or H3-K79 methylation has no effect on H2B ubiquitination, suggesting that the cross-talk is unidirectional. This control of a modification pattern in '*trans*' is site-specific since another site of methylation of H3 in yeast, K36, is not affected [33•] (note: methylation of H3-K27 has not been detected in budding yeast [38]).

Interestingly, inter-histone cross-talk may not be restricted to a single nucleosome. In yeast, about 5% of H2B is estimated to be ubiquitinated [32••,37], about 35% of the total H3 pool is thought to be methylated on K4 [32••], and 90% of all H3 is methylated on K79 [39••]. Since ubiquitination of H2B appears to be far sub-stoichiometric to the methylation of H3, the newly discovered control mechanism might serve as a paradigm for 'master control switches' directing the modification pattern of a whole nucleosomal region (Figure 2b).

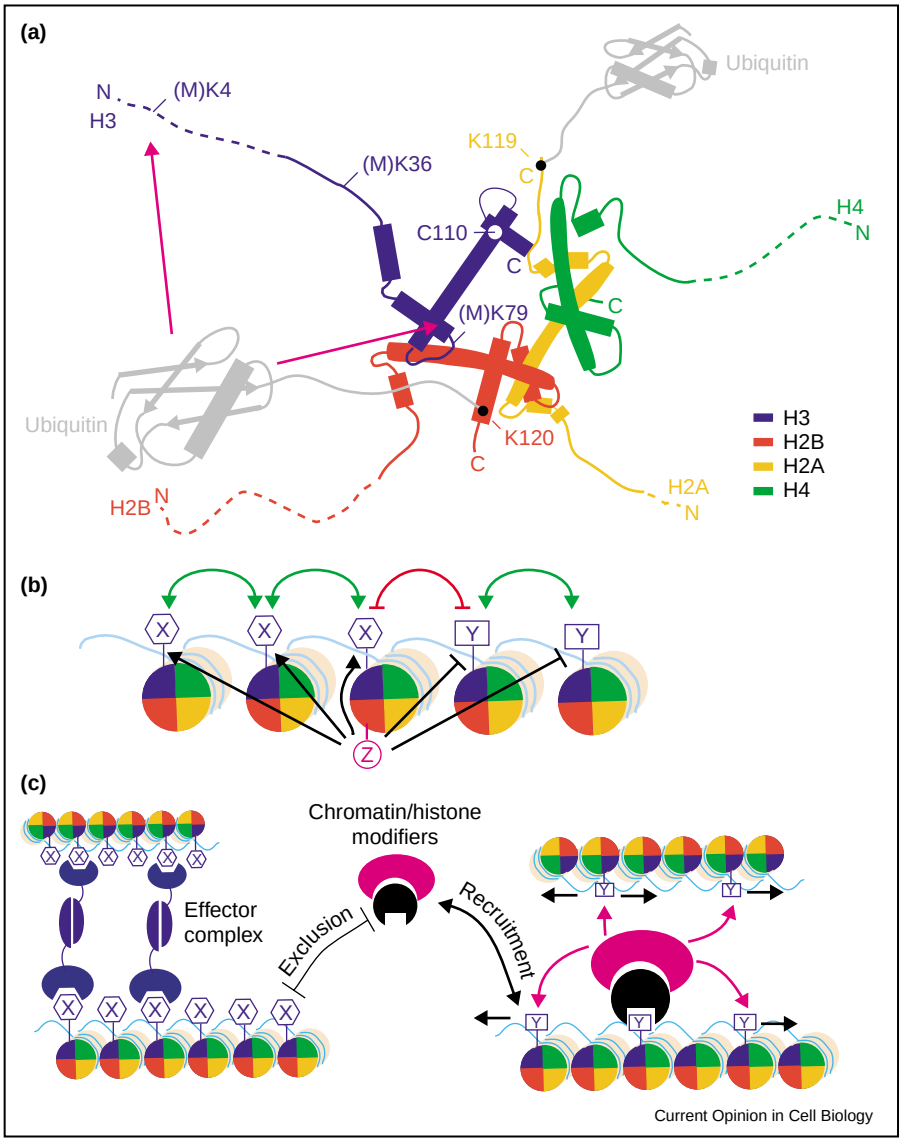
Another remarkable feature about this '*trans*-communication' is the cross-talk between distinct regions of the histone proteins: the amino-terminal tail (H3-K4), the histone core region (H3-K79) and the carboxy-terminal tail (H2B-K123) (see Figure 2a). So far, methylation on H3-K79 is the only known site of modification identified that lies within the nucleosome core domain (see Figure 1b; [39••,40•]). However, additional sites of modification in the globular region of H3 or other core histones may exist. Genetic studies in *S. cerevisiae*, for example, have identified two patches of sequence in the globular regions of H3 and H4 that are crucial for gene-silencing mechanisms and heterochromatin formation [41••]. In the crystal structure of the nucleosome, these regions are located at the H3/H4 histone-fold motif centered around H3-K79 (see Figure 1b,c). Whether other, yet-unknown, modifications in these patches provide additional cross-talk for the establishment of distinct chromatin readouts is an intriguing possibility.

Besides cross-talk between different covalent modifications, another way of 'communication' within the nucleosome core could be disulfide-bond-mediated dimerization. It may not be a coincidence that H3 is the only core histone containing a single cysteine (C110), which is conserved in all species except for budding yeast. Formation of a disulfide bond between the two H3 molecules of each nucleosome might place severe conformational restraints on the structure of individual nucleosomes, nucleosomal arrays or chromosomal domains (see Figures 1b and 2a for the positioning of C110 within H3 and a nucleosome, respectively). Early pioneering studies using iodoacetamide labeling have indicated that disulfide-linkage of H3 via C110 correlates with transcriptional silencing [42]. Nucleosomes in active regions, by contrast, might be actively maintained in a more reduced, and presumably more open, state. Such reduced regions overlap with hyperacetylated nucleosomes as indicated by mercury-column chromatography [43].

### Readout of histone and chromatin cross-talk

Singular as well as combinatorial histone modifications obviously impact on chromatin organization and structure. How is a specific modification pattern then translated into changes in genome status and activity? Modifications could directly interfere with the integrity and stability of a single nucleosome or an array of nucleosomes. Bulk acetylation, for example, has been shown to have the following effects: to alter the secondary structure of the histone tail; to weaken interactions between the histone tail and DNA; and to reduce internucleosomal interactions and chromatin folding (see [44] for references). These effects seem to result directly from changes in the net charge of the histone tails upon acetylation rather than from the presence of the actual mark. Besides biophysical experiments, genetic studies — for example on the acetylation of the histone variant H2A.Z in

**Figure 2**




Cross-talk at the level of individual nucleosomes and nucleosomal domains. **(a)** Schematic representation of four core histones (one copy of each H2A [orange], H2B [red], H3 [blue], and H4 [green]) as seen in the context of a nucleosome (residues are numbered according to the sequences of human histones). The dashed lines represent the unstructured tails. Mono-ubiquitination (gray) of the H2A and the H2B carboxy-terminal tails (K119 in H2A and K120 in H2B) is shown. In budding yeast, only H2B is known to be ubiquitinated (H2B-K120 of the human sequence corresponds to H2B-K123 in this organism). In a 'trans-tail' pathway, this modification is necessary for methylation of H3 on K4 and K79 (red arrows), but not K36 (see text for details). A conserved cysteine in H3 (C110) is indicated (white dot). **(b)** In an array of nucleosomes, different modifications on separate histones (X or Y) might influence each other in a positive or negative way. For example, it has been postulated that methylation of H3 on K9 could be spread over larger domains by recruitment of an HP1-Su(var)3-9 complex to sites of H3-K9 methylation (positive 'communication' X <=> X) [24,53]. Similarly, boundaries for modification spreading could be established by inhibition/exclusion of different modifications (negative 'communication' X >=< Y). On another level, a single modification could regulate the modification pattern of a larger region of nucleosomes ('master control switch', Z). Ubiquitination of H2B in budding yeast could be such a 'master control switch' because of its relatively low abundance in comparison with the methylation on H3-K4 and H3-K79, which are both dependent on this modification (see text). Since histone ubiquitination might be less stable than histone methylation, it is also possible that ubiquitin is removed after a methylation event on the same nucleosome. **(c)** Chromatin cross-talk might be mediated and read by different mechanisms. Effector modules and histone-modifying complexes could be recruited by certain marks but excluded/repelled by other modifications. Effectors or effector complexes that contain more than one recognition module for a certain modification (or modification pattern) could mediate long-range effects. Such binding factors could serve as 'bridging clamps' to bring together and potentially anchor distant nucleosomal arrays. In addition, modifying enzymes that contain binding modules or bind to effectors could reinforce and expand the modification pattern to adjacent nucleosomes (chromatin/histone modifiers).

*Tetrahymena* [45] and of the H4 tail in *S. cerevisiae* [46] — support such a global readout of this modification via direct effects on nucleosome and chromatin structure (see also [47]).

However, other studies have shown that the biological effects of certain distinct marks appear to rely more on specific local binding factors. This docking of effectors to post-translationally modified chromatin is reminiscent of the modular interactions in other signaling pathways (see for example the recruitment of SH2 domains to phosphotyrosines; for references see [10]). Bromodomains (brm [brahma]-like domains) are present in several HATs and chromatin remodeling proteins, as well as in the general transcription factor TAF250, and bind acetylated lysines (for review see [48]). Sequential recruitment and anchoring of bromodomain-containing factors and complexes to the promoter region is indeed crucial for the activation of some genes [49•,50].

Proteins containing certain chromatin-organization modifier (chromo)domains, on the other hand, have been predicted to have affinity for methylated lysines [51]. In fact, heterochromatin protein 1 (HP1) can bind to methylated H3-K9 [52–54], and more-recent work suggests that the silencing protein Polycomb (Pc) might bind methylated H3-K9 and/or methylated H3-K27 [38,55,56]. It will be interesting to determine if other chromodomain-containing proteins bind yet other sites of lysine methylation in histones or, potentially, in non-histone proteins. Considering the enormous variability of histone modifications, it is likely that several other recognition modules still await discovery. For example, it is not known what docking modules, if any, bind to phosphoserines/phosphothreonines, methyl-arginines, and so on, in the context of histones.

Conversely, certain histone modifications or modification patterns appear to rather prevent the binding of chromatin-associated mediators or effector modules (see Figure 2c). Such 'exclusion/repulsion' has been shown in the case of methylation of H3 on K4, which results in reduced binding of a chromatin repressor complex to the H3 tail [57,58]. In addition, methylation of H3-K4 may inhibit the recruitment of repressive factors such as Pc and HP1 to H3-K9-methyl (see above) [59]. These findings are consistent with the notion that methylation of H3 on K4 is generally believed to be an activating mark in higher organisms. Similarly, it has been suggested that methylation of H3-K79 in budding yeast prevents the spreading of silenced heterochromatic regions by preventing the binding of silencing proteins/complexes such as Sir2 to nucleosomes [39••].

The readout of complex patterns consisting of more than one modification could require multiple distinct binding sites in one effector or in multiprotein complexes. Complex patterns could also first be translated into simpler patterns. For example, enzymes or enzyme systems that discriminate between certain modification states of their substrates in '*cis*' or '*trans*' could establish single nucleosomal marks that could then be read by singular modules (see the previous section on cross-talk at the level of single histone tails, and Figure 2c). Since additional sites of covalent histone modification are still being discovered, and more and more enzyme systems responsible for generating and maintaining these marks are being identified, it seems likely, if not certain, that many more examples of cross-talk between histone modifications and its readout will emerge.

## DNA–histone cross-talk in the establishment of histone modification patterns

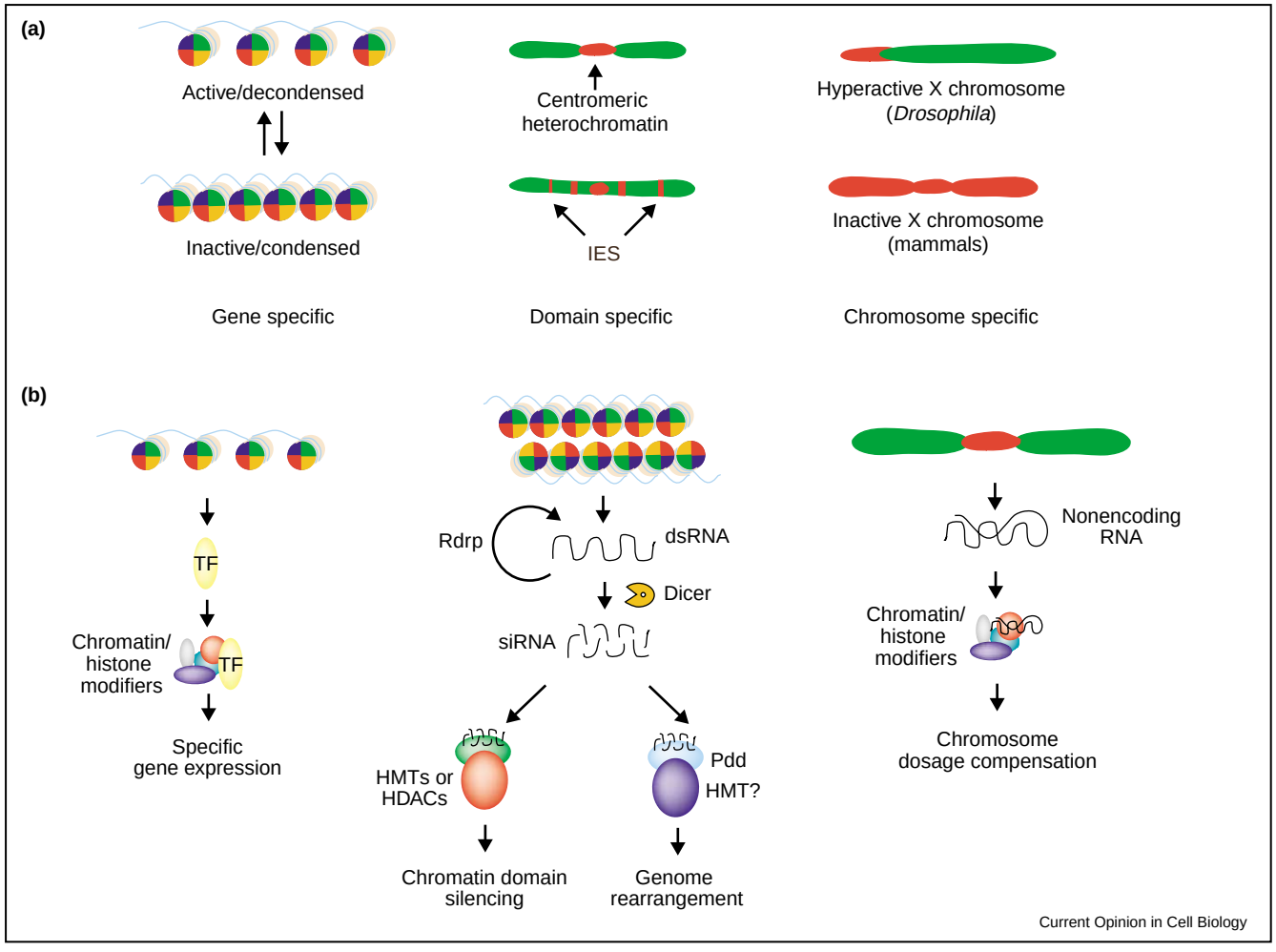
Singular and interlinked combinatorial histone modifications determine the chromatin status of small and large regions of the genome: locally, on the level of promoters and coding regions of genes; domain-restricted, on the level of euchromatic and heterochromatic regions of the genome; and globally, on the level of whole chromosomes (see Figure 3a). The establishment of specific modification patterns is initiated and controlled by cross-talk between specific DNA elements and histones.

### Local cross-talk

It is now established that local changes of chromatin structure are involved in the regulation of many if not all genes in eukaryotic cells [4]. Many DNA-sequence-specific transcription factors recruit enzymatic activities that post-translationally modify histones and/or chromatin to the promoter region of target genes [60] (Figure 3b). With the discovery of coactivator complexes containing HAT activity and co-repressor complexes containing HDAC activity, acetylation evolved as a paradigm of gene regulation by histone modifications [5,7,11]. Experiments using HDAC inhibitors as well as genome-wide ChIP approaches reflect a global dynamic equilibrium of histone acetylation. On this scale, acetylation and deacetylation reactions occur continuously, generating a steady-state level of global or bulk histone acetylation [61,62]. This equilibrium is locally perturbed by the recruitment of HATs and HDACs to promoter regions by site-specific transcriptional regulators. In response to environmental or developmental signals, these histone/chromatin-modifying activities are released or inactivated, allowing untargeted, globally acting enzymes to rapidly restore the steady-state levels of acetylation (within a time frame of minutes) [63•]. In *S. cerevisiae*, a direct impact of different HDACs on changes of local histone acetylation levels and on the transcriptional activity of targeted genes has been demonstrated on a genome-wide scale [64•].

In addition to local acetylation, promoter-restricted phosphorylation of histones has also been detected [8]. More

**Figure 3**




Histone modification cross-talk in chromatin and chromosome functions. **(a)** Histone and DNA modifications are involved in chromatin structure and function at multiple levels. Short-range cross-talk can determine the expression potential of particular genes by either influencing chromatin conformation or binding of chromatin-associated factors. At a higher level, combinatorial histone marks are likely to establish domains of chromatin. These specialized structures serve specific biological functions, such as the organization of centromeric heterochromatin, and the facilitation of chromosome rearrangement during *Tetrahymena* conjugation (elimination of IES, which resembles the VDJ recombination process in B/T-cell development in vertebrates). On a global scale, a unique pattern of histone modifications marks larger chromatin regions or, in some cases, whole chromosomes and controls their gene expression potential (e.g. dosage compensation). **(b)** Histone modifications and other chromatin components are engaged in cross-talk in several epigenetic regulatory pathways. On the single gene level, sequence specific transcription factors (TF, activating or repressive) are recruited to their target genes through protein–DNA interactions. Recent studies have found that the RNAi machinery is involved in establishing domains of specialized chromatin (Rdrp, RNA-dependent RNA polymerase; Dicer, dsRNA endonuclease). These regions include repressive heterochromatin at the mating type loci and the centromere in *S. pombe* and regions that most probably define IES during sexual conjugation of *Tetrahymena* (Pdd [programmed DNA degradation] proteins are specialized chromodomain-containing proteins involved in conjugation). Local diffusion of RNAs might contribute to the expandable nature of the domain structures. Non-coding RNAs (such as *roX1* and *roX2* in *Drosophila* and *Xist* in mammals) are crucially involved in the dosage compensation process of entire chromosomes. Obviously, different mechanisms have evolved for the selection of chromatin regions in local, domain-restricted, and global cross-talk. In all cases, histone and chromatin modifiers are recruited to establish distinct modification patterns for the control of genomic function. ds, double-stranded.

recently, it was shown that several transcription factors are able to recruit proteins or protein complexes containing HMT activity to specific sites of the genome ([65–67]. Similarly, changes in the level of promoter-associated histone methylation have been reported [25••,65, 68–70]. However, it is unclear if histone methylation is indeed reversible and the fate of methylated histones is currently unknown [1,20]. A functional interplay between histone acetylation, methylation of lysines and arginines, and phosphorylation in promoter-directed gene control is suggested from recent studies of the thyroid hormone receptor [68]. Importantly, *in vitro* experiments using reconstituted, chromatinized templates of single genes verify the impact that histones, and especially

histone tail domains and their post-translational modifications, have on local transcription units [71,72].

### Domain-restricted cross-talk

Histone modifications control not only local transcription units, but also larger regions of the genome. The chromosomes of higher eukaryotes are subdivided into discrete functional domains in which the expression of clustered genes is either favorable (euchromatin) or unfavorable (facultative heterochromatin). In addition, some gene-poor areas (such as the centromeric regions) are constitutively condensed (constitutive heterochromatin). By contrast, the genomes of lower eukaryotes (e.g. budding and fission yeast) are organized in a simpler way with heterochromatin-like areas restricted to relatively few genomic regions such as centromeres, telomeres and mating type loci [73]. With the exception of budding yeast, core histones, notably H3 and H4, in heterochromatin are generally hypoacetylated and methylated on H3-K9 [24,74]. By contrast, euchromatic regions largely appear to be hyperacetylated and methylated on H3-K4 [74]. Work using the polytene chromosomes of *Drosophila* has indeed indicated distinct banding patterns of histone modifications correlating with large transcriptionally competent or silenced regions [31,52,75,76].

Other studies indicate that marks defining heterochromatin are dominant over euchromatic marks and that insulating elements (boundaries and insulators) prevent the spreading of repressive histone modification patterns from one chromatin domain to another ([77,78]; and see [79,80] for further references). It is thought that boundary/insulator elements may organize the chromatin fiber into structurally different domains through the attachment of the DNA to a more-or-less fixed perinuclear substrate [81,82]. Although it remains unclear how insulating DNA elements of higher organisms exert their effects on the chromatin level, recent work in budding yeast supports a dynamic involvement of competing HAT and HDAC activities in maintaining the boundary at telomeric heterochromatin [83,84].

How then do certain regions of the genome direct the establishment of heterochromatic domains in the first place? One unifying feature of heterochromatic sequences is that they are highly repetitive and contain a large number of repeats and transposons [73]. Recent exciting work suggests that domain-restricted cross-talk between DNA and histones is mediated, in part, by small RNAs (Figure 3b). Studies in *Schizosaccharomyces pombe* link the expression of short double-stranded small heterochromatic RNAs (shRNAs) to the establishment of pericentric (constitutive) heterochromatin ([85••,86••]; see also [87–89]). These shRNAs are believed to be similar to small (~22–26 nt) RNAs involved in the gene-silencing RNA interference (RNAi) machinery. Indeed, deletion of genes homologous to components of the RNAi pathway of higher organisms in *S. pombe* impairs methylation of H3-K9 in centromeric heterochromatin, implicating that shRNAs might have a role in defining heterochromatin in this organism [86••]. Since heterologous repetitive DNA transferred to an ectopic site is sufficient for targeting H3-K9 methylation, it is conceivable that dsRNAs originating from pericentric repeats trigger the nuclear production of short RNAs, which in turn can induce formation of heterochromatin. Importantly, the RNAi machinery is only required for the initiation, but not the maintenance, of the heterochromatic state at the mating type loci [85••].

Support for an involvement of shRNAs in the cross-talk between DNA elements of low sequence complexity (i.e. high redundancy) and the establishment of histone modification patterns and chromatin domains comes from recent work in the ciliated protozoan *Tetrahymena thermophila*. The genome of this ciliate undergoes dramatic rearrangements by chromosome breakage and removal of internal eliminated sequences (IES) after sexual conjugation to form the macronucleus (transition of a germ cell nucleus to a somatic nucleus). Despite intensive studies, consensus DNA sequences marking the genomic regions to be eliminated could not be defined [90]. Interestingly, the DNA elimination process is impaired when Twi1, a gene involved in the production of small interfering RNA (siRNA) in other organisms, is disrupted [91••]. Indeed, production of siRNAs occurs during the time window of DNA elimination [91••]. Importantly, the eliminated chromatin domains are marked by H3-K9 methylation and hypoacetylation, whereas non-eliminated regions display the opposite pattern [92••].

Taken together, these findings implicate a conserved mechanism in the establishment of specialized heterochromatin domains that in turn govern processes as diverse as gene silencing and programmed DNA rearrangement. In both cases, cross-talk between genetic elements and histone modifications may be initiated by a combination of at least two potentially linked signals: a stretch of sequence-unspecific DNA repeats; and the local accumulation of aberrant shRNAs (see Figure 3b).

### Global cross-talk

Perhaps even more dramatic than the silencing of selective genomic domains by heterochromatin assembly is the inactivation of a whole chromosome. In female mammals, a single X chromosome is silenced during early embryogenesis in a stable and heritable fashion (for a review, see [93]). In contrast to the sequences of low complexity implicated in domain-restricted cross-talk discussed above, a unique locus, the X-inactivation center (*Xic*), directs the X-inactivation process, and controls the initiation and spreading of chromosome-wide gene silencing. Moreover, similar to the involvement of shRNAs in the silencing of genomic domains, an untranslated RNA (termed *Xist*) is the key mediator for cross-talk in

X-chromosome silencing (Figure 3b). Upregulation of *Xist* expression followed by global *Xist* coating of the chromosome that is going to be silenced are the earliest events in the X-inactivation process (a '*cis*'-limited effect) [94]. Intriguingly, the inactive X chromosome is consecutively globally methylated on H3-K9, suggesting that H3-K9 methylation is an early event in the formation of heterochromatin [95•,96•,97••,98•]. Once established, the inactive state is further enhanced by the hypoacetylation of H3 and H4 and the selective incorporation of the histone variant macro H2A in an *Xist*-independent manner [93].

Non-coding RNAs seem indeed to be a common theme in the control of larger chromosomal regions. In *Drosophila*, the single male X chromosome is transcriptionally hyperactivated (twofold upregulation; for review see [99]). Two partially redundant RNAs, called *roX1* and *roX2*, are central to the multistep hyperactivation process, which is regulated by the male-specific lethal (MSL) dosage-compensation complex. Production and local diffusion of these noncoding transcripts are responsible for X-chromosome-specific targeting and appear to mediate the nucleation of the dosage-compensation process (Figure 3b) [100]. Global gene hyperactivation is mediated by histone/chromatin-modifying activities in the MSL complex that establish patterns of H4-K16 acetylation and H3-S10 phosphorylation as part of this chromosome-wide gene regulation process.

## Conclusions and perspectives

The last decade has witnessed a revolution in molecular biology. The DNA sequences of several organisms have been largely annotated and chromatin has emerged as one, if not the key, regulator of genome function. Genetic regulatory mechanisms impact on chromatin on several different levels, directing the function of single genes, distinct chromosomal domains and, in some cases, whole chromosomes. A multitude of post-translational modifications of the main protein components of chromatin — histones — have now been identified. These result from distinct physiological stimuli and they in turn convey information that regulates the dynamics of the genome over the lifespan of a cell/organism. In contrast to the straightforward flow of most signal transduction cascades, where the modification of one protein impacts directly on downstream effectors, signaling to and from chromatin appears to be far more complex.

The first level of the complexity of chromatin cross-talk originates from the modular organization of chromatin itself. Each domain of chromatin contains a vast number of nucleosomes and each nucleosome contains two copies of each of the four core histone proteins. Obviously, each core histone can be post-translationally modified in a remarkably large number of ways, thus generating a vast number of possible combinations of marks for any chromatin domain. Besides a direct input from various signal transduction pathways on local and global chromatin levels, the modifications on single histones seem to be dependent on each other and to be interconnected via various mechanisms. Obviously, the complexity and diversity that are generated by the modification of chromatin add to the capacity of the genome to store, inherit and release information. We are only beginning to understand and appreciate the far-reaching implications of this non-DNA-encoded information for human biology and disease. Deciphering the many aspects of the proposed 'histone and chromatin cross-talk' represents a significant, but exciting, challenge.

## Acknowledgements and dedication

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During the course of preparing this manuscript, we were saddened to learn of the passing of Vincent G Allfrey, a true pioneer in the area of post-translational histone modifications and a great inspiration to the whole chromatin field. We dedicate this review to his memory.

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