Opinion

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Histone modification: cause or cog?

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Histone modifications are key components of chromatin packaging but whether they constitute a 'code' has been contested. We believe that the central issue is causality: are histone modifications responsible for differences between chromatin states, or are differences in modifications mostly consequences of dynamic processes, such as transcription and nucleosome remodeling? We find that inferences of causality are often based on correlation and that patterns of some key histone modifications are more easily explained as consequences of nucleosome disruption in the presence of histone modifying enzymes. We suggest that the 35-year-old DNA accessibility paradigm provides a mechanistically sound basis for understanding the role of nucleosomes in gene regulation and epigenetic inheritance. Based on this view, histone modifications and variants contribute to diversification of a chromatin landscape shaped by dynamic processes that are driven primarily by transcription and nucleosome remodeling.

An embroidery of chromatin modifications

There is general agreement in the chromatin field (see Glossary) that histone modifications play important roles in biological regulation. For example, acetylation of histone tails neutralizes the positive charge of lysines and profoundly alters chromatin properties [1,2]. Methylation of particular lysines on histone tails can increase the affinity of binding modules present on a variety of proteins that are thought to act by altering chromatin packaging [3]. Ancient roles for these and other modifications in chromatin transactions are thought to be responsible for the nearly universal amino acid sequence conservation of unstructured histone tails [4]. In addition, there are now many examples in which disruption of the histone modification machinery is associated with physiological alterations and disease [5–7], probably owing to their roles in transducing signals from the cellular environment to the genome [8]. Therefore, elucidating the mechanisms whereby histone modifications might be involved in cellular regulation is of central importance in understanding eukaryotic biology and in fighting disease. However, the complexity of chromatin, and the incomplete understanding of dynamic processes, mean that researchers remain mostly ignorant about how histone modifications contribute to eukaryotic gene regulation and chromosome packaging.

Over the past decade, the concept of a 'histone code', whereby combinations of modifications lead to important downstream events [4,9], has been a popular paradigm for trying to make sense of histone modification complexity. This concept has resulted in the adoption of metaphors such as 'writers' and 'readers', which are now commonplace in publications on histone modifications [10]. Additional terms imply causality, such as 'activating' or 'repressive' 'marks' [11]. In our view, these terms are inaccurate and misleading (Box 1). The question of causality is crucial both for understanding eukaryotic biology and for therapeutic intervention, so that the assumption of causality implicit in these terms needs to be rigorously established. Although in a handful of cases, histone modifications have been shown to be involved in particular transcriptional processes [5–7], the central premise of the histone code, that modifications are instructive, lacks experimental support. Enzymes that catalyze histone modifications must ultimately rely on

Glossary

Chromatin: genomic DNA in its packaged form, consisting primarily of nucleosomes, but also including linker histones, DNA-binding proteins and other protein complexes directly or indirectly bound to nucleosomes or linker DNA.

Chromodomain: a protein module that has evolved to recognize a methylated lysine on a histone tail. For example, the HP1 chromodomain preferentially binds H3K9me with increasing affinity for mono- to di- to tri-methyl, whereas the Polycomb chromodomain preferentially binds H3K27me.

Hidden Markov Model: a general machine-learning method that defines 'states' (e.g. nine chromatin states), together with probabilities of going from one state to the same state or to a different state. Statistical methods are used to calculate these 'transition probabilities' to obtain a best fit to the experimental data.

Histone modification: a covalent post-translational change to a histone residue, including lysine acetylation, methylation and ubiquitylation, serine phosphorylation, arginine methylation and many others, each catalyzed by one or more protein-modifying enzymes, many of which also have non-histone substrates.

Histone variant: a histone that is not encoded by the canonical histone genes. Whereas canonical histones are produced during S-phase for rapid incorporation behind the replication fork, histone variants are generally replication independent in their assembly and so can replace canonical histones throughout the cell cycle.

Nucleosome: the repeating subunit of chromatin that consists of an octameric core comprising two copies each of histones H2A, H2B, H3 and H4 or their variants, together with the approximately 147 base pairs of DNA that wrap around the core.

Nucleosome occupancy: the frequency with which a nucleosome is found at a given position in a population of cells. Incomplete occupancy implies that a nucleosome is either evicted or slid away from a position to account for its absence in some cells and presence in others.

Nucleosome position: the average genomic location of the midpoint of a nucleosome in a population of cells. Highly positioned nucleosomes show little variation in midpoint location, whereas 'fuzzy' nucleosomes are more randomly positioned.

SET domain: a protein module that catalyzes methylation of lysines on histone tails and on other proteins, often with high specificity, both *in vitro* and *in vivo*. For example, Set1 specifically catalyzes methylation of H3K4, and Set2 is specific for H3K36, despite the fact that both enzymes are associated with RNA Polymerase II and so are in the same milieu when encountering their H3 nucleosomal substrate.

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Box 1. Histone code semantics

Whether histone modifications constitute a 'code' has been the subject of seemingly endless debates [9]. Codes are central to the field of cryptography, and the conceptual basis for the Morse code is understood by the general public. In the Morse code, a telegraph key converts a piece of information into another representation. For example, 'e' is represented by one dot, and 'i' is represented by two dots. Likewise, the genetic translation machinery converts base triplets into single amino acids following the genetic code. For histone modifications to constitute a bona fide code, there must be a conversion key in which distinct combinations of histone modifications are converted into defined outputs. However, despite years of searching, we are aware of no verified example in which this ideal has been achieved. In addition, unlike the genetic code, where AUG encodes methionine, there is no comparably succinct, context-independent key that ties any particular histone modification or combination thereof to any distinct outcome. Other popular semantic spin-offs of the histone code also stack up poorly against the genetic code:

- The genetic code terms 'transcription' and 'translation' are, respectively, accurate metaphors for the action of RNA polymerase and for the combined action of ribosomes, tRNAs and aminoacyl tRNA synthases, which move in a continuous manner over a string of nucleotide bases.
- The histone code terms 'writers' and 'readers' are poor metaphors for what these proteins do. Writers do not write, but only modify one amino acid residue at a time. Likewise, readers do not read, they only bind, also one residue at a time. Calling them modifiers and binders, respectively is accurate and avoids the overstated metaphor.
- The terms 'activating' and 'repressive' imply causality, thus mistaking correlation for causation. As a result of this fallacious terminology, not-so-exceptional cases in which activating marks are found to be associated with repression and repressive marks with activation are thought to be remarkable.

sequence-specific transcription factors or small RNAs for targeting their action to promoters, enhancers and other regulatory elements [12]. In addition, although these enzymes are sometimes required for epigenetic inheritance, the histone marks that they lay down do not self-propagate [13,14].

Here, we argue that key histone modifications are better understood as cogs in the machinery that regulates transcriptional elongation, heterochromatic silencing and other processes. We find that the considerably older DNA accessibility paradigm [15] provides a more useful description of the dynamic chromatin landscape and is supported by recent findings.

Do combinations of modifications dictate chromatin states?

At about the same time that a histone code was proposed to exist [4], genome-scale methods for mapping histone modifications were introduced [16,17], and there are now dozens of 'ChIP-chip' and 'ChIP-seq' studies that profile histone modifications at an increasingly greater resolution and genome coverage [18–21]. When coupled with sophisticated computational techniques, these data sets can be used to predict sites of regulatory elements and the more data sets that are used, the more robust the predictions [22]. For example, a machine-learning method called a 'Hidden Markov Model' (HMM) was used to predict features of the *Drosophila* chromatin landscape [23]. Based on patterns of 18 histone modifications mapped genome-wide, the model defined nine distinct chromatin states that segmented the genome into thousands of regions of variable size. For example, transcriptional start sites were found to be especially abundant in H3K4me2, H3K4me3 and H3K9ac, and other combinations were found to be enriched in regions of transcriptional elongation, developmental silencing and constitutive heterochromatin. Similar analyses of mammalian cell data have achieved comparable success [24–26]. Although impressive, the ability to predict key attributes of the chromatin landscape does not necessarily imply that the modifications, either singly or in combination, are themselves involved in establishing or maintaining the features that they predict. To conclude that would be to accept the classic fallacy that correlation implies causation. However, even if histone modifications do play functional roles in defining these features, it is not clear which of the many modifications that contribute to the computational models are causally involved, and in which of the many genomic segments the modifications play an active role. Although the application of machine-learning algorithms to multiple histone modification data sets can provide fine distinctions in the chromatin landscape, model complexity can obscure mechanistic understanding. Complex states based on such abstract models are difficult for biologists to understand and experimentally test, and so their practical utility relative to simpler representations of chromatin states [27] remains to be demonstrated.

Genetic strategies can potentially distinguish cause from consequence, for example by mutation, deletion or knockdown of genes encoding histone-modifying enzymes or by enzyme inhibition using small molecules. However, because these enzymes can have non-histone substrates, and it is generally not feasible to distinguish direct from indirect effects, such approaches are only suggestive at best. The situation is further complicated by the fact there are often multiple enzymes that are specific for single modified residues, such as the MLL and Trithorax families of SET domain proteins that specifically methylate H3K4 [7]. A more direct approach is to change the modifiable amino acid. For example, changing the four acetylatable lysines on the H4 tail to arginine in all possible combinations resulted in gene expression changes [28], consistent with a role for histone H4 acetylation in modulating gene expression. However, in most cases, it did not seem to matter which combinations of the four lysines were changed to arginine, but rather the total number of lysines predicted differences in gene expression levels between the mutants. This finding is consistent with the notion that neutralization of basic lysine residues by acetylation reduces interactions of histone tails with neighboring nucleosomes, thus affecting the physical properties of nucleosomes [2,29] (Box 2).

Because complex eukaryotes have many copies of each canonical histone gene, often in multiple locations, it has not been feasible to replace histones with mutant versions to test whether their modifications are involved in developmental processes. *Xenopus* oocytes represent an exception, because they can be injected with large amounts of histone mRNAs, a strategy used by Ng and Gurdon to investigate the role of chromatin in epigenetic memory

Box 2. What is the relationship between histone acetylation and gene expression?

Histone modifications do not seem to be essential for transcription, because budding yeast lacking either the H3 or the H4 N-terminal tail are viable [64]. Nevertheless, overall acetylation levels are thought to be important for normal levels of expression [28,65]. Whether these effects of histone modifications on transcriptional levels reflect their direct action on the transcriptional machinery, as opposed to indirect effects, is unknown. Furthermore, the relationship between histone acetylation and gene expression is not clear cut. For example, in human T-cells, there are as many genes enriched for histone acetyltransferases (HATs) as for histone deacetylases (HDACs) and, contrary to expectation, HDACs are not enriched at epigenetically silenced genes [66]. The dynamic nature of acetylation–deacetylation cycles suggests that acetylation of histone tails functions to permit chromatin transactions transiently [1]. In *Drosophila*, acetylated nucleosomes turn over rapidly, are enriched for ATP-dependent nucleosome remodelers and display DNAseI hypersensitivity [23], suggesting a role for acetylation–deacetylation cycles in maintaining accessibility of regulatory DNA. In this way, a histone mark is neither 'activating' nor 'repressive', but rather it facilitates nucleosome dynamics.

during early embryonic development [30]. By injecting an excess of mRNA encoding the histone 3 replacement variant, H3.3, carrying a lysine-4 to glutamic acid mutation, they found that memory was all but lost in mutant embryos. Recent advances in genetic engineering technologies [31] promise to overcome the technical challenge of replacing multiple histone genes, and to enable rigorous testing of roles of histone modifications and variants in developmental processes.

The chromatin landscape is dynamic

Revolutionary technological advances in genomics over the past several years have begun to define the chromatin landscape at single nucleosome resolution, sometimes with single base-pair precision [18,32]. The picture that is emerging from these studies is that the chromatin landscape is more complex than the simple traditional concept of 'open' and 'closed' chromatin implies. In addition, progress in understanding the action of enzymes that act on chromatin, including ATP-dependent nucleosome remodelers and RNA polymerases, underscores the importance of dynamic processes in shaping the chromatin landscape. Much of this progress comes from studies of budding yeast, where genetic manipulability and a simple small genome has made it a favorite model system for asking mechanistic questions about the relationship between chromatin and transcription, and similar advantages of fission yeast have led the way in understanding silencing mechanisms [33]. Although yeast chromatin is gene rich, and might not be an ideal model for higher-order chromatin structure, these findings in yeast have been generalized to multicellular eukaryotes, including humans. Similar to patterns of 'active' histone modification, distinct patterns of nucleosome occupancy and positioning are characteristic of transcriptional activity and provide highly detailed landscapes that can be used to predict regulatory elements [34–36] (Figure 1). Therefore, post-translational modifications are superimposed upon a complex nucleosomal landscape, so that a full understanding of how histone modification patterns are generated

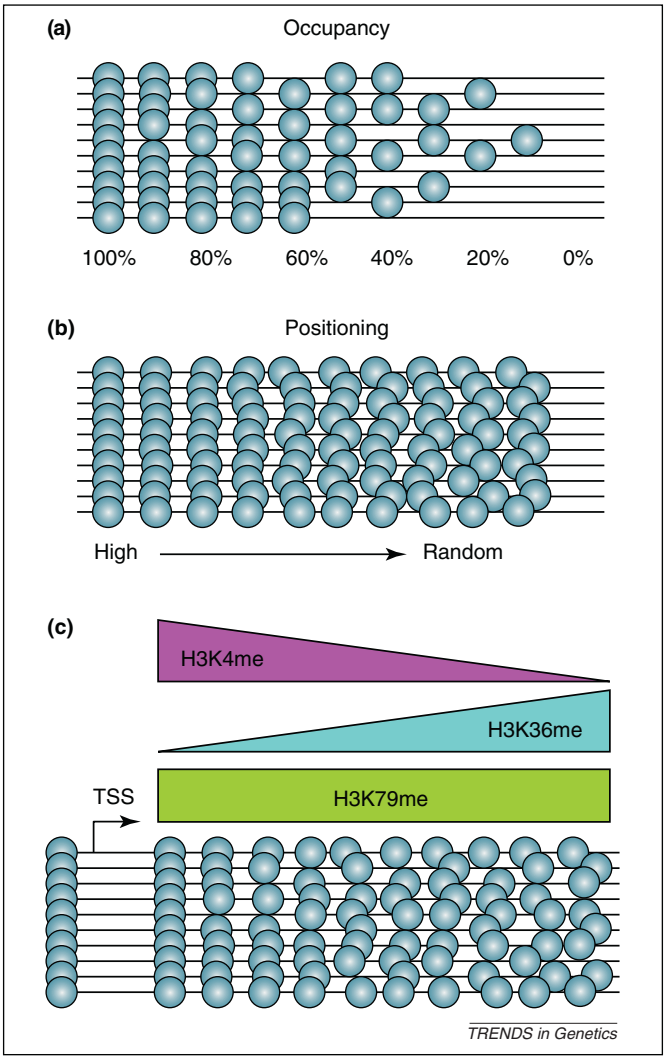

Figure 1. Histone modifications superimposed on a complex and dynamic nucleosome landscape. Cartoon depiction of nucleosome occupancy **(a)** and positioning **(b)**, where parallel lines represent ten precisely aligned genomes and nucleosomes are shown as grey balls. Reduced occupancy implies nucleosome turnover in a homogeneous population of cells [61], whereas differential positioning implies regulated nucleosome mobility [62]. **(c)** Examples of histone modifications that are enriched over transcription units, showing gradients for H3K4me, which is enriched near transcriptional start sites, and for H3K36me, which becomes enriched farther from the 5′ ends of genes [37]. H3K79me is relatively uniform over gene bodies. The cartoon below shows average patterns of occupancy and positioning of the type seen in genic regions, where occupancy and positioning are typically high around 5′ ends of genes and positioning drops off farther downstream of the transcriptional start site (TSS). Adapted from [34].

will require knowing the availability of the target amino acid at the time that it is being acted upon by a modifying enzyme. Although knowledge of these states is far from complete, recent high-resolution nucleosome mapping studies have uncovered some general principles.

One principle that has emerged from mapping nucleosomes is that their positioning in and around transcription units varies depending on their distances from 5′ and 3′ ends of genes (Figure 1). There is an ongoing debate as to the extent to which positioning is determined directly by sequence or by the action of ATP-dependent remodelers and RNA polymerase [34], with profound implications for the generation of histone modification patterns. For histone modifications that change with alterations in gene activity, correlations between their patterns and the extent

of nucleosome positioning might simply be consequences of nucleosome disruption during transcription and remodeling. For example, patterns of mono-, di- and tri-methylation of H3K4 are most easily understood as resulting from tethering of the COMPASS histone methyltransferase complex to RNA polymerase during active transcription [37,38] (Box 3). In this way, the positioning of a nucleosome over a site represents the degree to which it resists being mobilized, either passively by sequence or actively by transcriptional or remodeling processes. Another principle is that nucleosome occupancy at a site can differ over the entire range, from sites that are so depleted of nucleosomes that common mapping methods cannot detect them to sites that appear to be fully occupied (Figure 1). In a homogeneous population of cells, partial occupancy implies that nucleosomes are evicted and reassembled, because a position that is partially occupied by a nucleosome must be occupied in some genomes in a population and unoccupied in others. Thus, the degree of occupancy at a position can be interpreted in terms of nucleosome stability, where stable nucleosomes show full occupancy and highly unstable nucleosomes showing depletion. In support of this interpretation, recent studies indicate that many so-called 'nucleosome-free' regions are probably partially or transiently occupied *in vivo*, but the histone cores are lost during standard chromatin preparation procedures [39,40]. Thus, nucleosome positioning and occupancy are measures of nucleosome mobility and stability, respectively.

DNA accessibility as a paradigm for chromatin regulation

Nucleosomes block access of many DNA-binding proteins to their sites of action, and accessible regions are those that regulate gene expression, initiation of DNA replication origins and other DNA transactions. This concept was put forward by Weintraub and Groudine 35 years ago in their introduction of DNaseI hypersensitivity mapping [15], which is still a widely used method for determining sites of heightened steady-state accessibility of DNA to strand cleavage [41,42]. More recent high resolution nucleosome mapping studies provide unequivocal verification of this seminal concept. For example, the yeast *PHO5*

Box 3. Transcription-coupled generation of histone modification patterns

In organisms as diverse as yeast and humans, gene activity is associated with gradients of histone modifications that show a 5′-to-3′ polarity [37]. For example, all three levels of H3K4 methylation gradually decrease over gene bodies, with the trimethylation gradient closest to the promoter, followed by dimethylation enrichment, followed by the monomethylation enrichment. An opposite gradient of enrichment is seen for H3K36 methylation. These successive patterns and gradients can be explained by the association of the Set1 histone lysine methyltransferase with the Ser-5 phosphorylated initiation form of the RNA Pol II C-terminal domain [38] (Figure I). Likewise, the Set2 protein associates with the Ser-2 phosphorylated elongating form of the RNA Pol II CTD. H3K4me and H3K36me are examples of conserved modifications that are highly correlated with active transcription, but they are not necessarily 'activating'.

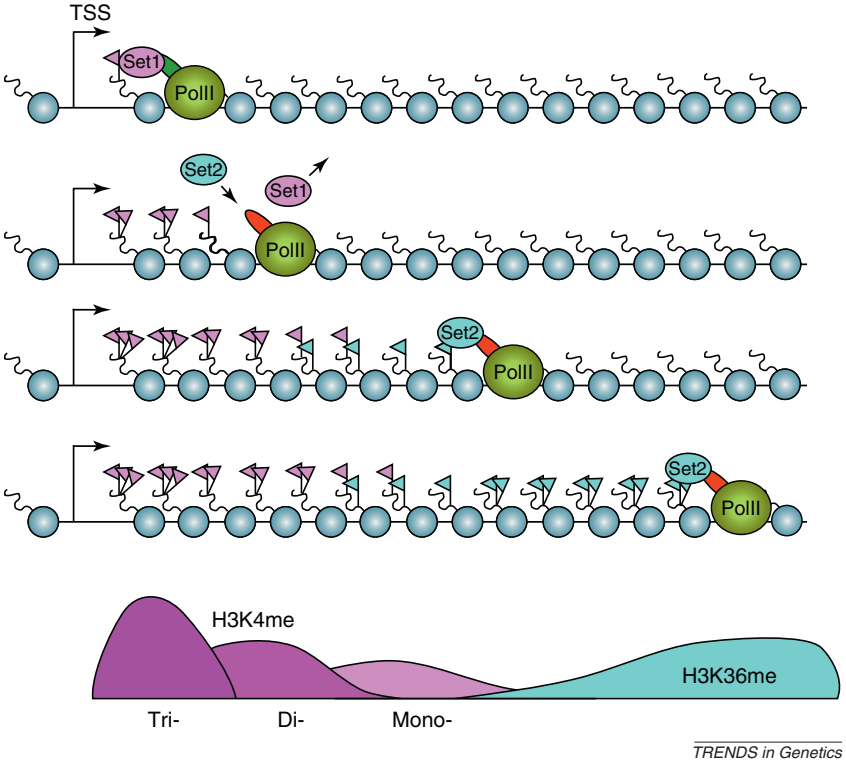

Figure I. A model for the transcription-coupled generation of histone modification patterns. In the cartoon, the H3 tail (squiggly line) is methylated by either Set1/COMPASS (magenta flags) at lysine-4 or by Set2 at lysine-36 (cyan flags). In this way, the association of a modifying enzyme with a transiting RNA polymerase exposes histone tails to the enzyme while it disrupts each nucleosome (grey ball) that it encounters. This process results in distinct modification patterns that decrease with distance from the 5′ end of an actively transcribed gene [37].

promoter is occupied by nucleosomes in the off state, and these nucleosomes are evicted upon induction by phosphate [43,44]. Blocking of promoters by nucleosomes is also a hallmark of gene silencing in cancer. For instance, the promoter of the *MLH1* mismatch repair gene is occupied by nucleosomes when silenced in various cancers, but becomes reactivated and unblocked following treatment with a drug that causes loss of DNA methylation [45]. It does not appear that loss of DNA methylation by itself is sufficient to reactivate gene expression, but rather it is the resulting eviction of nucleosomes that enables transcription factors to access the *MLH1* promoter for it to function. In this way, DNA methylation is thought to help stabilize nucleosomes and thus maintain low accessibility of pro-

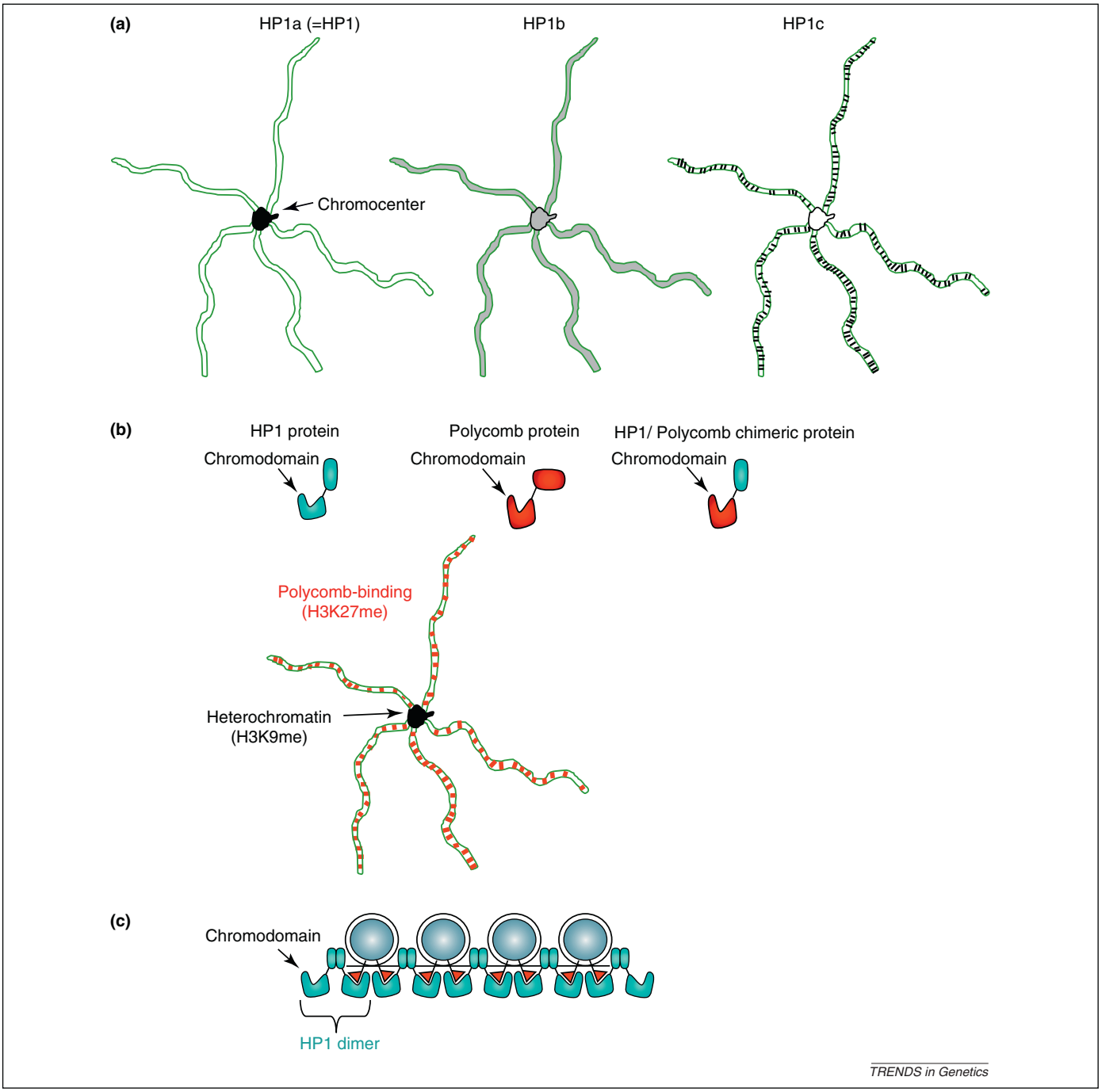

Figure 2. Histone tail modifications do not recruit chromodomain proteins, but instead are needed for them to bridge neighboring nucleosomes. **(a)** Closely related *Drosophila* heterochromatin-associated protein 1 (HP1) paralogs display different localization patterns. HP1a localizes to the heterochromatic chromocenter of the giant salivary gland polytene nucleus [67], whereas HP1b is found at both heterochromatin and euchromatin and HP1c is found at transcriptionally active sites within euchromatin [51,68]. Swap experiments showed that the 'hinge' region and the chromoshadow domain impart targeting specificity, whereas swapping only the chromodomain had no effect. **(b)** *Drosophila* HP1 is targeted by protein–protein interactions. Platero *et al.* [52] replaced the chromodomain of HP1 with that of Polycomb protein, and used polytene chromosome immunolocalizations to assay the localization of both the chimeric HP1/Polycomb protein and both endogenous proteins. The chimera localized to both the chromocenter and Polycomb binding sites (red bars on euchromatic arms). Remarkably, both HP1 and Polycomb endogenous proteins also became localized to both classes of sites, despite the fact that HP1 sites are marked by H3K9me and Polycomb sites by H3K27me, which were later shown to be the specific moieties bound by their chromodomains. **(c)** *In vitro* studies of *Schizosaccharomyces pombe* HP1 (Swi6) indicate that it forms dimers that bridge pairs of methylated H3K9 residues on nearby nucleosomes. Adapted from [53].

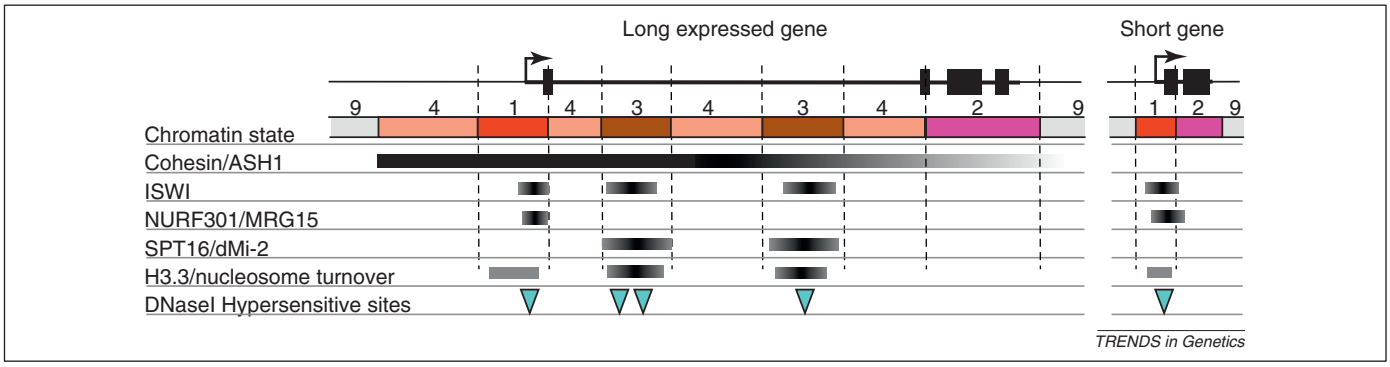

Figure 3. Complex histone modification patterns correspond to simple measurements of nucleosome dynamics and occupancy. A Hidden Markov Model (HMM) was used to define nine chromatin states based on 18 histone modifications [23]. For example, state 1 (red bar) was most frequently found over promoters of active genes, state 2 over exons and state 3 over introns. States 1 and 3 were also enriched for ATP-dependent nucleosome remodeling proteins (ISWI, NURF301 and dMi-2), for transcriptional elongation factors (MRG15 and SPT16), and for nucleosome turnover, both as measured by H3.3 histone variant patterns and by CATCH-IT, a direct measure of nucleosome turnover kinetics [14]. States 1 and 3 were also enriched for discrete sites of DNaseI hypersensitivity [42]. By contrast, Cohesin and ASH1, which are more generally associated with transcriptionally active chromatin, displayed a much broader distribution. Reproduced, with permission, from [23].

moters and other functional elements. DNA methylation can change the physical properties of nucleosomes [46], although the molecular basis for enhanced stability is unknown.

DNA methylation is maintained epigenetically by semi-conservative replication, which results in long-term stabilization of nucleosomes and the consequent inaccessibility of regulatory elements, an important mechanism for silencing genomic parasites, such as transposable elements [47]. It is likely that the so-called 'repressive' modifications also stabilize nucleosomes, although the mechanisms whereby these marks act and how they are maintained through development is unclear. Constitutively repressive chromatin is mediated by the heterochromatin-associated protein 1 (HP1), a dimer with 'chromodomains', each of which binds H3K9me3 with micromolar affinity [48]. It is often asserted that modification of a histone recruits proteins that contain binding modules, such as chromodomains, but given the weak binding affinities of modules for modified residues, it is likely that modifications only help to stabilize assembled complexes [48–50]. In fact, targeting of chromodomain-containing proteins was found not to depend on chromodomain specificity. For example, only HP1a (=HP1), not its two closely related paralogs within the *Drosophila* genome, localizes to heterochromatin [51] (Figure 2a). HP1b localizes throughout the genome, whereas HP1c localizes to regions of active chromatin, with localization specificity residing outside of the chromodomain. Furthermore, targeting of both HP1 and the Polycomb chromodomain-containing protein depend on protein–protein interactions, rather than on specific histone modifications [52] (Figure 2b). An attractive model for HP1 action in the cell is that it helps to stabilize nucleosomes and so favor heterochromatin formation by bridging nearby H3 tails that carry this modification [53] (Figure 2c). Nucleosome stabilization might also explain the presence of HP1 in bodies of active genes [54,55], where it would help prevent the loss of nucleosomes that are disrupted during RNA polymerase transit. HP1 binds *in vivo* with a residence time of a few minutes [56,57], which might be long enough to reduce nucleosome eviction and maintain silent heterochromatin in a compacted state. Compaction is also thought to underlie developmental silencing [58,59], perhaps by preventing nucleosome eviction [14]. There might be analogous examples in which active chromatin is maintained by the binding of modification-specific modules, and indeed there are many examples of such modules on proteins associated with active chromatin, including bromodomains, PhD fingers, Tudor domains and others [60]. It seems probable that the affinity of a binding module for a modification is a factor in the modulation of nucleosome dynamics. However, the molecular mechanisms whereby binding of these modules to modifications can affect gene expression are not clear.

Because there are so many different modules on various key chromatin regulators, it is difficult to decide which modifications and which interactions might be responsible for any downstream effect. It might be that each such interaction has a partial effect on the stability or mobility of a nucleosome, and much of the complexity of the system is the cumulative result of many such interactions, involving nucleosome remodelers, RNA polymerase, histone chaperones, histone modifying enzymes and chromatin-associated proteins [61].

Whether histone modification patterns primarily reflect the action of dynamic processes that alter DNA accessibility is unknown. Nevertheless, it is intriguing that 'active' modification patterns revealed by applying an HMM to data from 18 histone modifications corresponded closely to patterns obtained by mapping of ATP-dependent nucleosome remodelers, DNAseI hypersensitivity sites and histone turnover [23] (Figure 3). It is unclear to us whether such a complex model based on combinations of modifications is better able to predict regulatory elements than what could be obtained simply by using DNA accessibility or turnover data. Although we are excited by the promise of improved epigenomic mapping technologies for understanding biological regulation, it remains to be determined which chromatin features will be the most informative.

Concluding remarks

Describing histone modifications in terms of information or language suggests overwhelming complexity and leaves mechanistic questions unaddressed. By contrast, DNA accessibility provides a simple testable paradigm for understanding the role of nucleosomes in gene regulatory

processes. The view that emerges is that dynamic processes affecting nucleosomes result in patterns of histone modifications, which in turn affect the physical properties of nucleosomes and help to maintain the active or silent state of chromatin [61,62]. Such dynamic processes include not only transcription factor binding, RNA polymerase elongation and nucleosome remodeling, but also the targeting action of long non-coding RNAs [63]. A fuller understanding of how these dynamic processes result in histone modification patterns will be needed to evaluate the functional significance of histone modifications in gene expression and other chromatin-associated processes.

Acknowledgments

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References

1 Waterborg, J.H. (2002) Dynamics of histone acetylation *in vivo*. A function for acetylation turnover? *Biochem. Cell Biol.* 80, 363–378
2 Shogren-Knaak, M. *et al.* (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844–847
3 Fischle, W. *et al.* (2003) Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* 17, 1870–1881
4 Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* 403, 41–45
5 Cao, R. and Zhang, Y. (2004) The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr. Opin. Genet. Dev.* 14, 155–164
6 Bungard, D. *et al.* (2010) Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. *Science* 329, 1201–1205
7 Smith, E. *et al.* (2011) The super elongation complex (SEC) and MLL in development and disease. *Genes Dev.* 25, 661–672
8 Turner, B.M. (2011) Environmental sensing by chromatin: an epigenetic contribution to evolutionary change. *FEBS Lett.* 585, 2032–2048
9 Gardner, K.E. *et al.* (2011) OPERating ON Chromatin, a colorful language where context matters. *J. Mol. Biol.* 409, 36–46
10 Ruthenburg, A.J. *et al.* (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol. Cell* 25, 15–30
11 Hublitz, P. *et al.* (2009) Mechanisms of transcriptional repression by histone lysine methylation. *Int. J. Dev. Biol.* 53, 335–354
12 Enderle, D. *et al.* (2011) Polycomb preferentially targets stalled promoters of coding and noncoding transcripts. *Genome Res.* 21, 216–226
13 Ptashne, M. (2007) On the use of the word 'epigenetic'. *Curr. Biol.* 17, R233–R236
14 Deal, R.B. *et al.* (2010) Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* 328, 1161–1164
15 Weintraub, H. and Groudine, M. (1976) Chromosomal subunits in active genes have an altered conformation. *Science* 193, 848–856
16 Iyer, V.R. *et al.* (2001) Genomic binding sites of the yeast cell–cycle transcription factors SBF and MBF. *Nature* 409, 533–538
17 Ren, B. *et al.* (2000) Genome-wide location and function of DNA binding proteins. *Science* 290, 2306–2309
18 Zhang, Z. and Pugh, B.F. (2011) High-resolution genome-wide mapping of the primary structure of chromatin. *Cell* 144, 175–186
19 Zhou, V.W. *et al.* (2011) Charting histone modifications and the functional organization of mammalian genomes. *Nat. Rev. Genet.* 12, 7–18
20 Liu, C.L. *et al.* (2005) Single-nucleosome mapping of histone modifications in *S. cerevisiae*. *PLoS Biol.* 3, e328
21 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
22 Wang, Z. *et al.* (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* 40, 897–903
23 Kharchenko, P.V. *et al.* (2011) Comprehensive analysis of the chromatin landscape in *Drosophila melanogaster*. *Nature* 471, 480–485
24 Rada-Iglesias, A. *et al.* (2011) A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* 470, 279–283
25 Ernst, J. and Kellis, M. (2010) Discovery and characterization of chromatin states for systematic annotation of the human genome. *Nat. Biotechnol.* 28, 817–825
26 Hon, G. *et al.* (2009) Discovery and annotation of functional chromatin signatures in the human genome. *PLoS Comput. Biol.* 5, e1000566
27 Filion, G.J. *et al.* (2010) Systematic protein location mapping reveals five principal chromatin types in *Drosophila* cells. *Cell* 143, 212–224
28 Dion, M.F. *et al.* (2005) Genomic characterization reveals a simple histone H4 acetylation code. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5501–5506
29 Megee, P.C. *et al.* (1995) Histone H4 and the maintenance of genome integrity. *Genes Dev.* 9, 1716–1727
30 Ng, R.K. and Gurdon, J.B. (2008) Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat. Cell Biol.* 10, 102–109
31 Gunesdogan, U. *et al.* (2010) A genetic system to assess *in vivo* the functions of histones and histone modifications in higher eukaryotes. *EMBO Rep.* 11, 772–776
32 Floer, M. *et al.* (2010) A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. *Cell* 141, 407–418
33 Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* 301, 798–802
34 Pugh, B.F. (2010) A preoccupied position on nucleosomes. *Nat. Struct. Mol. Biol.* 17, 923
35 Lee, C.K. *et al.* (2004) Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* 36, 900–905
36 Wang, X. *et al.* (2011) An effect of DNA sequence on nucleosome occupancy and removal. *Nat. Struct. Mol. Biol.* 18, 507–509
37 Rando, O.J. (2007) Global patterns of histone modifications. *Curr. Opin. Genet. Dev.* 17, 94–99
38 Krogan, N.J. *et al.* (2003) The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell* 11, 721–729
39 Weiner, A. *et al.* (2010) High-resolution nucleosome mapping reveals transcription-dependent promoter packaging. *Genome Res.* 20, 90–100
40 Xi, Y. *et al.* (2011) Nucleosome fragility reveals novel functional states of chromatin and poises genes for activation. *Genome Res.* 21, 718–724
41 Shibata, Y. and Crawford, G.E. (2009) Mapping regulatory elements by DNaseI hypersensitivity chip (DNase-Chip). *Methods Mol. Biol.* 556, 177–190
42 Hesselberth, J.R. *et al.* (2009) Global mapping of protein–DNA interactions *in vivo* by digital genomic footprinting. *Nat. Methods* 6, 283–289
43 Reinke, H. and Horz, W. (2003) Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. *Mol. Cell* 11, 1599–1607
44 Lin, J.C. *et al.* (2007) Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. *Cancer Cell* 12, 432–444
45 Adkins, M.W. *et al.* (2007) Chromatin disassembly from the PHO5 promoter is essential for the recruitment of the general transcription machinery and coactivators. *Mol. Cell. Biol.* 27, 6372–6382
46 Choy, J.S. *et al.* (2010) DNA methylation increases nucleosome compaction and rigidity. *J. Am. Chem. Soc.* 132, 1782–1783
47 Yoder, J.A. *et al.* (1997) Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet.* 13, 335–340
48 Jacobs, S.A. and Khorasanizadeh, S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* 295, 2080–2083
49 Vermeulen, M. *et al.* (2007) Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* 131, 58–69
50 Vermaak, D. and Malik, H.S. (2009) Multiple roles for heterochromatin protein 1 genes in *Drosophila*. *Annu. Rev. Genet.* 43, 467–492
51 Smothers, J.F. and Henikoff, S. (2001) The hinge of and chromo shadow domain impart distinct targeting of HP1-like proteins. *Mol. Cell. Biol.* 21, 2555–2569

52 Platero, J.S. *et al.* (1995) Functional analysis of the chromo domain of HP1. *EMBO J.* 14, 3977–3986
53 Canzio, D. *et al.* (2011) Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly. *Mol. Cell* 41, 67–81
54 Vakoc, C.R. *et al.* (2006) Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol. Cell. Biol.* 26, 9185–9195
55 Braunschweig, U. *et al.* (2009) Histone H1 binding is inhibited by histone variant H3.3. *EMBO J.* 28, 3635–3645
56 Festenstein, R. *et al.* (2003) Modulation of Heterochromatin Protein 1 dynamics in primary mammalian cells. *Science* 299, 719–721
57 Cheutin, T. *et al.* (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 299, 721–725
58 Francis, N.J. *et al.* (2001) Reconstitution of a functional core polycomb repressive complex. *Mol. Cell* 8, 545–556
59 Eskeland, R. *et al.* (2010) Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol. Cell* 38, 452–464
60 Yap, K.L. and Zhou, M.M. (2010) Keeping it in the family: diverse histone recognition by conserved structural folds. *Crit. Rev. Biochem. Mol. Biol.* 45, 488–505
61 Henikoff, S. (2008) Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat. Rev. Genet.* 9, 15–26
62 Cosgrove, M.S. *et al.* (2004) Regulated nucleosome mobility and the histone code. *Nat. Struct. Mol. Biol.* 11, 1037–1043
63 Wang, K.C. *et al.* (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 472, 120–124
64 Ling, X. *et al.* (1996) Yeast histone H3 and H4 amino termini are important for nucleosome assembly *in vivo* and *in vitro*: redundant and position-independent functions in assembly but not in gene regulation. *Genes Dev.* 10, 686–699
65 Kurdistani, S.K. *et al.* (2004) Mapping global histone acetylation patterns to gene expression. *Cell* 117, 721–733
66 Wang, Z. *et al.* (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 138, 1019–1031
67 James, T.C. *et al.* (1989) Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*. *Eur. J. Cell Biol.* 50, 170–180
68 Kwon, S.H. *et al.* (2010) Heterochromatin protein 1 (HP1) connects the FACT histone chaperone complex to the phosphorylated CTD of RNA polymerase II. *Genes Dev.* 24, 2133–2145