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Signalling through Chromatin Modifications and Protein–Protein Interactions

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Introduction

Compaction of the eukaryotic genome into chromatin inside the nucleus is essential for faithful segregation of mitotic chromosomes to daughter cells without deleteriously tangling the DNA strands. To begin with, nuclear DNA is wrapped around core histones H2A, H2B, H3, and H4 to form nucleosomes (Luger, 2003; Khorasanizadeh, 2004). Association of linker histones with the DNA connecting adjacent nucleosomes leads to the higher order 'solenoid' or 30 nm helical chromatin fibre. The 30 nm fibre is the prevalent form of interphase chromosomes and is the stadium on which a variety of nuclear activities take place. On the other hand, packing DNA into chromatin restricts the access to DNA by many regulatory

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Abbreviations: ACTR, activator of nuclear receptor; CARM1, coactivator-associated arginine methyl transferase; CBP, CREB binding protein; Chromodomain, chromatin organization modifier; CoREST, corepressor RE1-binding silencer protein; CREB, cAMP response element binding protein; CTD, carboxy terminal domain; Dnmt1, human DNA cytosine-5 methyltransferase; E(Z), enhancer of zeste; FAD, flavin adenine dinucleotide; GNAT, GCN5-related N-acetyltransferases; HAT, histone acetyl transferase; HDAC, histone deacetylase; HMT, histone methyl transferase; HP1, heterochromatin protein 1; LSD1, lysine-specific demethylase; MMS, methyl methanesulfonate; MOF, males absent on the first; MYST, MOZ-Ybf2/Sas3-Sas2-Tip60; NAD, nicotinamide adenine dinucleotide; NuA3/4, nucleosome acetyltransferase of histone H3/H4; PAD, protein arginine demethylases; Pc, polycomb; PCAF, P300/CBP associated factor; PHD, plant homeodomain; PRC1, polycomb repressor complex 1; PRMT1, protein arginine methyl transferase; PTB, phosphotyrosine binding; RSC, remodel the structure of chromatin; SAGA, Spt-Ada-Gcn5 acetylase; SAM, S-adenosyl methionine; SET, Su(var), E(z) trithorax; SH2, Src homology 2; SIR2, silent information regulator 2; SMN, survival motor neuron; SRC-1, steroid hormone receptor co-activator 1; SUMO, small ubiquitin-related modifier; SUV39H1, suppressor of variegation 3–9 homologue; SWI/SNF, switching-defective/sucrose non-fermenting; TAF1, TATA associated factor 1; TBP, TATA binding protein; UbLs, ubiquitin-like proteins.

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factors involved in such functions as replication, transcription, DNA repair, and recombination. Dynamic changes in the local and global organization of chromatin are thus key to regulating genomic functions (Wolffe, 2001; Kamakaka, 2003). Conserved mechanisms that counterbalance the repressive nature of chromatin and allow greater access to DNA include: (1) chromatin-remodelling complexes that contain ATPase subunits capable of sliding, replacing, or altering histone–DNA interactions (Cairns, 2005); (2) covalent modifications of the histone tails, such as acetylation, methylation, phosphorylation, and ubiquitinylation; and (3) incorporation of histone variants into the nucleosomal particles (Henikoff and Ahmad, 2005).

This review will focus on histone modifications and their roles in regulation of chromatin functions. Proteins that are recruited to specifically modified histones are described briefly.

Histone acetylation

Reversible acetylation in the amino terminal domains of the core histones correlates positively with transcriptional activation, recombination, and repair (Hebbes *et al.*, 1988; Kuo *et al.*, 1996; Grunstein, 1997; Struhl, 1998; Kundu *et al.*, 2000; Eberharter and Becker, 2002; Carrozza *et al.*, 2003). Over the years, multiple histone acetyltransferases (HATs) and deacetylases (HDACs) have been identified (Roth *et al.*, 2001). A net local balance between the activities of these HATs and HDACs thus determines the acetylation state of chromatin. This functional interplay is fundamental to regulation in growth and developmental processes, and deregulation of such has been linked to the progression of different types of cancers (e.g. leukaemia, colorectal and breast cancer) and diverse human disorders, like the Rubinstein–Tabi (Petrij *et al.*, 1995) and fragile X syndromes (Timmermann *et al.*, 2001).

Several families of HATs have been recognized: GNAT (Gcn5-related N-acetyltransferases); MYST (MOZ-Ybf2/Sas3-Sas2-Tip60); p300/CBP (although the catalytic domains of p300 and CBP are very similar to those of the GNAT family HATs); nuclear hormone coactivators; and TAF1 (formerly TAFII230 in human and TAFII250 in *Drosophila*) (Roth *et al.*, 2001). To date, the yeast Gcn5 is the best characterized of the HATs, both structurally and functionally and both *in vivo* and *in vitro* (Candau *et al.*, 1997; Kuo *et al.*, 1998; Wang *et al.*, 1998). *In vitro*, recombinant Gcn5 can acetylate histone H3 strongly on lysine 14 and H4 on lysine 8 and 16 (Kuo *et al.*, 1996). In humans and mice, the Gcn5 subclass of acetyltransferases is represented by two closely related proteins, GCN5 and p300/CREB-binding, protein-associated factor (PCAF) (Xu *et al.*, 1998b). HAT and coactivator functions of PCAF have been demonstrated in myogenesis (Puri *et al.*, 1997) and nuclear receptor-mediated (Chen *et al.*, 1997; Blanco *et al.*, 1998; Leo and Chen, 2000) and growth factor-signalled activation (Xu *et al.*, 1998a). Structural and kinetic studies of GNAT and MYST families of HATs have been thoroughly executed (Marmorstein and Roth, 2001). From yeast to mammals, many HATs form complexes with other proteins. In the budding yeast *Saccharomyces cerevisiae*, there are several chromatographically distinct HAT complexes displaying specific preference for histones and lysine residues (Grant *et al.*, 1997, 1998). The SAGA (Spt-Ada-Gcn5 acetylase) and NuA3 complexes prefer H3, while NuA4 uses nucleosomal H4 as the predominant substrate. Compared to the yeast complexes, mammalian HAT complexes are

less thoroughly characterized. PCAF is also part of a complex whose composition is quite similar to that of SAGA (Ogryzko *et al.*, 1998). Both SAGA and PCAF complexes contain selective TBP-associated factors (TAFs). Another GNAT family member, Hat1, initially shown to be responsible for the predominant cytoplasmic HAT activity in *S. cerevisiae*, can acetylate, in association with Hat2, lysine-12 of the histone H4 N-terminal tail region (Parthun *et al.*, 1996; Ruiz-García *et al.*, 1998). Elp3 is capable of acetylating all four core histones and is involved in transcriptional elongation (Wittschieben *et al.*, 1999).

p300 and CBP of higher eukaryotes are highly similar to each other throughout the entire length (~300 kilodaltons in size) (Ogryzko *et al.*, 1996). In many cases, p300 and CBP appear to perform redundant functions. However, animal models and patient studies suggest non-overlapping roles as well (Iyer *et al.*, 2004; Kalkhoven, 2004). SAGA, PCAF, and p300/CBP HATs are recruited to the target promoters by interacting with selective transcriptional activators (Roth *et al.*, 2001). Compared with other HATs, recombinant p300/CBP is exceptionally versatile in that it is able to acetylate all four histones within nucleosomes, as well as in free-histone form (Ogryzko *et al.*, 1996; Martínez-Balbás *et al.*, 1998).

The MYST family of HATs possesses in their catalytic domain an acetyl CoA binding motif and a C_2HC zinc finger (Carrozza *et al.*, 2003). In addition, some MYST family members contain a chromodomain, whereas others possess the PHD (plant homeodomain) finger that is also shared in many chromatin regulators (Aasland *et al.*, 1995). The chromodomain has been shown to interact with methylated lysines (see below). Consistent with the largely repressive functions of chromodomain proteins, at least two MYST proteins, Sas2 and Sas3, promote transcriptional silencing at HM mating type loci (Ehrenhofer-Murray *et al.*, 1997) and at telomeres (Reifsnyder *et al.*, 1996) in yeast. Another MYST family protein, Esa1, is an essential HAT for cell cycle progression (Clarke *et al.*, 1999). Some MYST HAT complexes exert their activity across a much larger chromosomal locus. For example, in *Drosophila*, the male insect has only one X chromosome, while the female has two. To compensate for the dosage difference, male insects express X chromosomal genes twice as efficiently as each of the two female X. This dosage compensation requires acetylation of Lys16 of H4 across the male X chromosome by MOF (Lucchesi, 1998; Akhtar and Becker, 2000; Birchler *et al.*, 2003).

There are three major families of HDACs (Grozinger and Schreiber, 2002): Class I and II members are each homologous to the yeast Rpd3 and Hda1 proteins, respectively. These enzymes require a zinc ion and a water molecule for catalysis, and they are sensitive to several HDAC inhibitors, some of which are promising cancer therapeutic agents (Drummond *et al.*, 2005). On the other hand, the Class III HDACs are homologues of the yeast Sir2 protein. These HDACs are NAD-dependent enzymes insensitive to the inhibitors for the other two classes of HDACs (Marmorstein, 2001, 2004). The Sir2-related Class III HDACs, because of their obligatory need of NAD for catalysis, have been linked to intracellular sensing of nutrient/energy status and senescence (Blander and Guarente, 2004; Guarente, 2005). Interestingly, the product of deacetylation by Sir2 family enzymes is 2'-O-acetyl-ADP-ribose, which has been suggested to be a novel secondary messenger. Unique activators and inhibitors for Sir2-related deacetylases were recently reported (see Denu, 2005 for a review).

Many HDACs function within large complexes that are recruited to specific loci by DNA-binding transcriptional repressor proteins (Robyr *et al.*, 2002). In addition to deacetylating histones and other proteins, HDACs also collaborate with other enzyme activities, most notably histone and DNA methyltransferases, such as Suv39h and Dnmt1, respectively (Jepsen and Rosenfeld, 2002; Narlikar *et al.*, 2002). Recent data showed that a lysine demethylase, LSD1/BHC110/AOF2, is part of the BHC/BRAF–HDAC complex that represses neuronal-specific genes (Shi *et al.*, 2004; Lee *et al.*, 2005; Metzger *et al.*, 2005).

Histone methylation

Although methylation of histones was first documented four decades ago (Murray, 1964), very little was known about the biological consequences of this covalent modification until recently. For a long time, it was also believed that histone methylation was more of an irreversible modification that could not be removed in a cell division-independent manner (Byvoet *et al.*, 1972; Duerre and Lee, 1974). This view was compatible with its role in transcriptional silencing, such as those found at centromeric heterochromatin, DNA-methylated promoters or as epigenetic marks for the inheritance of the silenced chromatin (Zhang and Reinberg, 2001; Kouzarides, 2002). Nevertheless, the existence of a process that reverses histone methylation is certainly necessary when considering the role of methylation in cases of regulated and dynamic gene expression (Chen *et al.*, 1999; Strahl *et al.*, 1999; Rea *et al.*, 2000; Bannister *et al.*, 2002). Both lysine and arginine residues can be methylated. Methylated lysines can be found either in a mono-, di-, or trimethylated state (Rice *et al.*, 2003), whereas methylated arginine can be in a mono- or di-modified state. Dimethylated arginine may be symmetrical or asymmetrical, depending on the positions of these two methyl groups. Unlike acetylation, methylation of the ε-amino group of lysines in the amino-terminal tails of histones does not alter the overall charge of the histone tails; however, increasing the number of methyl groups (mono, di or tri) does increase its basicity and hydrophobicity. The net result is thus a perceivably stronger association between methylated histone tails and DNA/chromatin. Methylation of histones is catalysed by histone methyltransferases (HMTs), and all known HMTs utilize S-adenosyl-methionine (SAM) as the methyl group donor. HMTs can be grouped into two divergent families: histone lysine methyltransferases catalysing the methylation of lysine residues (Lachner and Jenuwein, 2002; Sims *et al.*, 2003), and protein arginine methyltransferases (PRMTs) that catalyse the methylation of arginine residues (Stallcup, 2001).

Well-characterized methyl lysines of histones include K4, K9, K27, K36, K79 of H3, and K20 of H4 (Feng *et al.*, 2002; Lacoste *et al.*, 2002; Ng *et al.*, 2002b; van Leeuwen *et al.*, 2002). The mammalian Suv39h enzymes and their *Schizosaccharomyces pombe* homologue, Clr4, were the first histone lysine methyltransferases (HMTases) identified (Rea *et al.*, 2000; Nakayama *et al.*, 2001). The conserved SET domains in these proteins catalyse methylation of H3 lysine 9 that in many systems is correlated with transcriptional repression and silencing. Human and mouse genomes encode over 50 predicted SET-domain proteins (Kouzarides, 2002), while *S. pombe* has about 10 putative SET domain HMTases. Interestingly, H3 lysine 4 instead of lysine 9 is the predominant methylated site observed in *S. cerevisiae* that has seven

SET domain proteins (Briggs *et al.*, 2001). Most fully activated promoters are enriched in tri-methylated H3-K4 (Briggs *et al.*, 2001; Santos-Rosa *et al.*, 2002), whereas basal transcription correlates well with H3-K4 dimethylation (Wang *et al.*, 2001a). Histones H3-K9, H3-K27, and H4-K20 methylation are all hallmarks of condensed chromatin state, and recruitment of several H3-K9-specific HMTases causes gene repression within euchromatin (Nielsen *et al.*, 2001; Tachibana *et al.*, 2001; Nishioka *et al.*, 2002; Ogawa *et al.*, 2002).

A histone lysine-specific demethylase (LSD1) was reported recently, providing the most compelling evidence that lysine methylation is also a reversible and dynamic function (Shi *et al.*, 2004). Instead of cleaving the N-CH_3 bond directly, LSD1 induces amine oxidation of specifically mono- or dimethylated histone H3 lysine 4 to generate unmodified lysine and formaldehyde. A nuclear homologue of amine oxidases, LSD1, utilizes FAD as a cofactor and, together with HDACs, is a component of the CoREST and other corepressor complexes (Lunyak *et al.*, 2002; Lee *et al.*, 2005). A more recent finding revealed that LSD1 can relieve repressive histone marks by demethylation of histone H3 at lysine 9 (H3-K9), thereby leading to de-repression of androgen receptor target genes (Metzger *et al.*, 2005). Although evolutionarily conserved from *S. pombe* to mammals, there appears to be no direct orthologue of this demethylase in *S. cerevisiae*, despite extensive H3 lysine 4 methylation (Santos-Rosa *et al.*, 2002). Allshire and colleagues recently proposed that fission yeast protein Epe1, and other JmjC domain-containing proteins, two of which are present in the budding yeast *S. cerevisiae*, may be putative histone demethylases that could act by oxidative demethylation to demethylate mono-, di- or trimethylated histones (Trewick *et al.*, 2005).

Methylation of arginine residues is associated with active transcription (Bannister *et al.*, 2002). CARM1 methyltransferase directs histone H3 Arg17 and Arg26 methylation in response to hormone induction, and cooperates synergistically with p160-type coactivators (e.g. GRIP1, SRC-1, ACTR) and coactivators with histone acetyltransferase activity (e.g. p300, CBP) to enhance gene activation by steroid and nuclear hormone receptors (Ma *et al.*, 2001; Bauer *et al.*, 2002). PRMT1 also facilitates transcriptional activation for nuclear receptors by methylating arginine 3 of H4 (Strahl *et al.*, 2001; Wang *et al.*, 2001b). Arginine methylation is susceptible to enzymatic turnover by de-imination reaction by protein arginine demethylases or PADs (Cuthbert *et al.*, 2004; Wang *et al.*, 2004a). Strictly speaking, de-imination is not a true reversal of methylation as it generates citrulline, instead of arginine, and methyl-ammonium. Comparatively, the responsible enzymes and functions of H4 Arg3 methylation in *S. cerevisiae* are much less understood (Lee *et al.*, 2000; Lacoste *et al.*, 2002).

Histone phosphorylation

Reversible protein phosphorylation is one of the most important and well-studied post-translational modifications. Phosphorylation plays critical roles in the regulation of many cellular processes including cell cycle, growth, apoptosis, and signal transduction pathways. Serine, threonine, and tyrosine are the major, but not exclusive, eukaryotic phosphorylation sites.

The SQ motif in the C-terminal tail of H2A of lower eukaryotes (S129 Q130) is

known to undergo rapid phosphorylation in response to DNA damage by γ-irradiation (Downs *et al.*, 2000; Redon *et al.*, 2003; Nakamura *et al.*, 2004). In higher eukaryotes, one H2A variant, H2A.X, also carries a similar motif at the same position relative to the stop codon (S139) that responds to DNA damage in a similar fashion (Rogakou *et al.*, 1998; Madigan *et al.*, 2002). The SQ motif is a good consensus site for the PIKK (phosphatidylinositol 3-kinase-like kinase) family of kinases, members of which are responsible for phosphorylation of this motif upon DNA damage in *S. cerevisiae* (Mec1 and Tel1) and higher eukaryotes (ATM, ATR, and DNA-PK) (Burma *et al.*, 2001; Durocher and Jackson, 2001; Shroff *et al.*, 2004; Stiff *et al.*, 2004). Chromatin immunoprecipitation experiments in budding yeast have demonstrated that phosphorylation of the SQ motif spreads up to 50–100 kb of chromatin from the lesion of an induced double-strand break (Downs *et al.*, 2004; Unal *et al.*, 2004). Several protein complexes are known to bind to the phosphorylated SQ motif (Stewart *et al.*, 2003; Ward *et al.*, 2003; Downs *et al.*, 2004; Kusch *et al.*, 2004; Morrison *et al.*, 2004; van Attikum *et al.*, 2004). Recruitment of HAT and ATP-dependent chromatin remodelling activities is believed not only to reorganize the chromatin structure at the site of damage, but also to expose it to other DNA damage response pathways or allow access to phosphatases to remove the phosphate on H2A or H2AX after the damage is repaired (Foster and Downs, 2005). In addition, DNA damage has also been linked recently to Ser1 phosphorylation of histone H4 in yeast by casein kinase II (Cheung *et al.*, 2005). Whether and how this modification recruits the relevant repair factors remains to be established.

Phosphorylation of Ser10 in histone H3 is linked to transcription and mitotic progression, two functions with opposite requirement for chromatin compaction: chromatin condensation during mitosis and relaxation for transcription (Hendzel *et al.*, 1997; Cheung *et al.*, 2000; Prigent and Dimitrov, 2003; Nowak and Corces, 2004). During chromatin condensation and segregation in mitosis in eukaryotes, Ser10 phosphorylation originates from the centromeric region and then spreads through entire chromosomes (Hendzel *et al.*, 1997; Wei *et al.*, 1999). In *Tetrahymena*, alanine substitution at H3 Ser10 causes defects in condensation and segregation (Wei *et al.*, 1998). On the other hand, mitotic condensation of *C. elegans* can proceed in the absence of H3 Ser10 phosphorylation (Speliotes *et al.*, 2000), suggesting the existence of a different mechanism for condensation/segregation, or functional redundancy of histone H3 phosphorylation. Indeed, mitotic phosphorylation of H3 also occurs at Ser28 (Goto *et al.*, 1999), Thr11 (Preuss *et al.*, 2003), and Thr3 (Shoemaker and Chalkley, 1980; Polioudaki *et al.*, 2004). In addition, one H3 variant, H3.3, also is phosphorylated at Ser31 during mitosis (Hake *et al.*, 2005).

Contrary to the global phosphorylation during mitosis, transient and gene-specific phosphorylation of H3 at Ser10 is observed following stimulation of mammalian cells with growth factors that cause activation of target genes (Herschman, 1991; Mahadevan *et al.*, 1991; Barratt *et al.*, 1994; Chadee *et al.*, 1999; Sassone-Corsi *et al.*, 1999; Salvador *et al.*, 2001). Also, transcription-related Ser10 phosphorylation was shown to be important for transcriptional induction of several yeast genes (Lo *et al.*, 2001, 2005). Besides growth factors, diverse stimuli such as phorbol esters, DNA damage by UV, or alkylating agents, and pharmacological compounds, can induce H3 phosphorylation (Mahadevan *et al.*, 1991) via activation of various kinase pathways. Phosphorylation of H3 Ser28 is also induced

by stress and UV irradiation (Zhong *et al.*, 2001, 2003) and has very similar kinetics to those of Ser10. Both serines lie in the same consensus sequence (-ARKS-) and are very likely modified by overlapping enzymes (Shibata *et al.*, 1990; Goto *et al.*, 1999, 2002; Sugiyama *et al.*, 2002), including mitogen activated protein kinases (MAPKs), extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases (JNKs), mitogen and stress-induced kinases 1 and 2 (MSK1/2), cAMP-dependent protein kinase (PKA), protein kinase C (PKC), IκB kinase α (IKKα), and p90 RSK2 (Zhong *et al.*, 2000; Bode and Dong, 2003, 2005; Clayton and Mahadevan, 2003; Yamamoto *et al.*, 2003).

For mitotic progression, Ser10 is mediated by Aurora kinases such as Ipl1p of *S. cerevisiae* (Wei *et al.*, 1999), Ark1 of *S. pombe* (Petersen *et al.*, 2001), and Aurora A, B, and C in higher eukaryotes (Glover *et al.*, 1995; Schumacher *et al.*, 1998; Hsu *et al.*, 2000; Adams *et al.*, 2001; Prigent and Giet, 2003). A very recent report demonstrated that defects associated with the Ipl1 yeast Aurora B kinase can be suppressed by deleting the Set1 histone methyltransferase (Zhang *et al.*, 2005), linking mitotic chromatin condensation to regulation of histone methylation. Casein kinase II catalyses histone H4 Ser1 phosphorylation in response to MMS- or phleomycin-induced double-stranded breaks (DSBs), and is important for non-homologous end joining (Cheung *et al.*, 2005). Furthermore, phosphorylation of mammalian histone H2B Ser14 (equivalent to Ser10 of *S. cerevisiae* H2B) is catalysed by sterile-20 (Ste20) kinase in response to apoptotic signals (Cheung *et al.*, 2003; Ahn *et al.*, 2005). Thus, histone phosphorylation is achieved by a variety of kinases from different signal transduction pathways. How different signals converge on and affect the structure and biophysical characteristics of chromatin will undoubtedly remain a hot research field for years to come.

Ubiquitinylation and sumoylation of histones

Ubiquitin (Ub) and small ubiquitin-like modifier, SUMO, are structurally highly conserved proteins that are covalently conjugated to target proteins through an isopeptide bond between their carboxy-terminal glycine and the ε-amino group of lysine residues in the substrate protein. Both types of modifications are achieved by the sequential and concerted action of the activating enzyme (E1), conjugating enzyme (E2), and ligase (E3) (Hochstrasser, 1996; Pickart, 2004). Furthermore, the internal lysine residue of ubiquitin can act also as the acceptor, leading to polyubiquitinylation. SUMO, on the other hand, is generally thought to function as a monomer, although polymeric forms of SUMO perform complex functions in higher eukaryotes (Bylebyl *et al.*, 2003; Li *et al.*, 2003). While the canonical view remains that the polyubiquitin chain acts as a general device targeting the underlying proteins for proteolysis by the 26S proteosome (Pickart, 2001), non-proteolytic functions of monoubiquitinylation have emerged in the recent past (Spence *et al.*, 2000; Hicke, 2001). Indeed, both ubiquitinylation and sumoylation have been identified as important mechanisms for cellular regulation of transcription, DNA repair, cell cycle progression, protein localization and trafficking (di Fiore *et al.*, 2003; Schnell and Hicke, 2003; Seeler and Dejean, 2003; Hay, 2005).

Interestingly, the first eukaryotic protein found to be ubiquitinylated was H2A from HeLa cells. Subsequently, histones H2B, H3 and H1, and the H2A.Z variant

were shown to carry this modification. In *S. cerevisiae*, however, H2B appears to be the sole histone that is ubiquitinylated. Ubiquitin moiety is added to a conserved lysine residue (lysine 123 in yeast and lysine 119 in vertebrate H2B) in the C-terminal tail that is accessible for interactions with DNA, adjacent nucleosomes, and other regulatory factors (Thorne *et al.*, 1987; Robzyk *et al.*, 2000). Yeast mutants deleted for a well-known Ub E2 enzyme, Rad6, have no detectable levels of H2B ubiquitinylation (Robzyk *et al.*, 2000). Recent reports identified Bre1 as the E3 enzyme that directs Rad6 to monoubiquitinylation of H2B (Hwang *et al.*, 2003; Wood *et al.*, 2003a). Besides harbouring the typical features of an E3 ligase, *bre1* deletion mutants have several phenotypes in common with a *rad6* deletion mutant or H2B K123R mutant. However, more recent findings seem to suggest that Bre1 may not be the only H2B ubiquitinylation E3 enzyme. Subunits of the PAF complex, previously implicated in transcription elongation, also function to regulate Rad6 activity in monoubiquitinylation of H2B (Ng *et al.*, 2003; Wood *et al.*, 2003b; Mueller *et al.*, 2004).

Several groups have uncovered a novel interplay of modifications involving H2B ubiquitinylation and H3 methylation. Deletion of *RAD6* or mutation of H2B ubiquitinylation site prevents H3 methylation at Lys4 and 79 (Dover *et al.*, 2002; Ng *et al.*, 2002a; Sun and Allis, 2002). On the other hand, Set2-mediated H3 lysine 36 methylation is unaffected (Briggs *et al.*, 2002). Models to explain this unidirectional regulation of H3 methylation by H2B ubiquitinylation have been proposed, which suggest that either the bulky ubiquitin moiety on H2B serves to unfold the chromatin to allow greater access to methyltransferases such as the Set1 and Dot1 (Briggs *et al.*, 2002; Henry and Berger, 2002), or might simply act as a tag recognized by proteins carrying ubiquitin interacting domains (Jason *et al.*, 2002). Alternatively, ubiquitinylated H2B might regulate the activity of specific H3 methyltransferases on chromatin, thereby promoting H3 methylation (Ng *et al.*, 2002b; Krogan *et al.*, 2003). While important for methylation, persistent H2B ubiquitinylation also may harm transcription. An ubiquitin hydrolase Ubp8 is found to be a stable component of the SAGA acetyltransferase and transcriptional coactivator complex (Henry *et al.*, 2003; Daniel *et al.*, 2004). Biochemical and genetic evidence indicates that Ubp8 targets H2B for deubiquitinylation. The dynamic balance of H2B ubiquitinylation/deubiquitinylation is important for *GAL1* transcription, since either substitution of the ubiquitinylation site in H2B (Lys123) or loss of Ubp8 lowers *GAL1* expression. Thus, unlike acetylation/deacetylation whose functions are mutually opposing in most cases, both ubiquitinylation and deubiquitinylation are required for gene activation. Intriguingly, the function of H2B (de)ubiquitinylation that involves a different ubiquitin hydrolase, Ubp10/Dot4, appears to be different for telomeric silencing (Emre *et al.*, 2005; Gardner *et al.*, 2005). The molecular mechanisms underlying such distinction remain to be elucidated.

In many higher organisms, 5–15% of histone H2A is ubiquitinylated at lysine 119. Until recently, the function of this modification and the factors involved in its establishment were unknown. Two recent papers, de Napoles *et al.* (2004) and Wang *et al.* (2004b), link monoubiquitinylation of histone H2A to the activities of E3 ubiquitin ligases that reside in Polycomb-group repressor complexes. Ubiquitinylated H2A (uH2A) occurs on the inactive X chromosome in female

mammals, and this correlates with the recruitment of the Polycomb repressor complex 1 (PRC1). The hPRC1L (human Polycomb repressive complex 1-like) is composed of several Polycomb-group proteins including Ring1, Ring2, Bmi1, and HPH2. Embryonic stem cells null for PRC1 components, Ring1B, and its close homologue, Ring1A, exhibit significant diminishment of global uH2A levels. In *Drosophila*, chromatin immunoprecipitation analysis demonstrated co-localization of dRing with ubiquitinylated H2A at the promoter of the *Drosophila* Ubx gene in wing imaginal discs. Removal of dRing in SL2 tissue culture cells by RNA interference results in loss of H2A ubiquitinylation and, concomitantly, derepression of Ubx. These studies thus provide evidence that H2A ubiquitinylation is a novel epigenetic marker for the inactive X chromosome (Xi) and link uH2A to Polycomb silencing.

Despite a very similar protein fold as revealed by the nuclear magnetic resonance (NMR) structure of SUMO-1 (Bayer *et al.*, 1998), the distribution of charged residues on the surface of SUMO is very different from that of ubiquitin or ubiquitin-like proteins (UbLs). These differences account for the facts that similar but distinct enzymes mediate SUMO conjugation and hydrolysis, as well as the unique functions associated with sumoylation. Recently, histone H4 was reported to be modified by SUMO (Shiio and Eisenman, 2003). Although the exact site(s) of sumoylation was not determined, the N-terminal tail was found to be the substrate for SUMO modification *in vitro*. Co-expression of histone acetyltransferase p300 enhances sumoylation of histone H4. It remains to be seen if sumoylation of H4 affects nucleosomal structure or modulates its interaction with other chromatin related factors. In the *Drosophila* polytene chromosomes, the SUMO moiety was detected in many euchromatic sites and the chromocentre (Lehembre *et al.*, 2000), suggesting a link to euchromatin functions. Future work should clarify the precise roles of histone/chromatin sumoylation in the regulation of chromatin function.

Modifications of histone variants

Diversification of core histone into variants contradicts our perception that histones indiscriminately package and compact the genome (Brown, 2001; Malik and Henikoff, 2003). Genes encoding the major histone proteins are often highly conserved, present in multiple copies, and expressed primarily during the S phase of the cell cycle. On the other hand, histone variants that differ from the core histones in their biophysical properties are restricted to specialized regions of the genome, and likely exist as single copy genes expressed throughout the cell cycle. Exchanging with the pre-existing histones during development and differentiation (Pina and Suau, 1987), histone variants offer specialized functions in regulating chromatin dynamics. The variants tend to differ from the major histones, particularly in the non-globular N- and C-terminal tail regions, and are involved in both transcriptional activation and repression. Some histone variants contribute to genome stability by regulating the fidelity of chromosome segregation or efficiency of DNA replication and repair (Kamakaka and Biggins, 2005). One obvious example is the centromere-specific histone H3 variant CENP-A in mammals and Cse4 in the budding yeast (Palmer *et al.*, 1991; Meluh *et al.*, 1998). Just as the core histones, the variants are also modified, which may aid their deposition or eviction out of the chromatin. H1

variant is phosphorylated in the tail during deposition and removal from chromatin (Dou *et al.*, 1999), H2A.Z is extensively acetylated (Ren and Gorovsky, 2001), while H3.3 is methylated and acetylated in a manner similar to the core histone H3 (McKittrick *et al.*, 2004). Phosphorylation at Ser31 of H3.3 was recently shown to localize at the pericentromeric region during mitosis (Hake *et al.*, 2005). It is almost certain that many exciting and insightful discoveries on histone variant modifications and functions will be reported in the near future.

Recruitment of proteins by chromatin modifications: case studies of bromodomain and chromodomain

How each of the many chromatin modifications elicits specific molecular functions is a critical and fascinating biological question. Accumulating evidence clearly shows that one of the mechanisms is the recruitment of selective proteins that interact directly with the corresponding histone modification. Two prominent examples are bromodomain and chromodomain, which interact respectively with acetylated and lysine methylated histones.

BROMODOMAIN

With the plethora of biological functions linked to acetylation (Carrozza *et al.*, 2003), it is very significant that the well-conserved bromodomain is capable of binding to acetyl lysine moieties (Dhalluin *et al.*, 1999; Jacobson *et al.*, 2000). The bromodomain (~60 amino acids) was first identified by sequence alignment of six genes from *Drosophila* (fsh and brm), yeast (*SPT7* and *SNF2*), and humans (CCG1 and RING3). It was later found conserved in almost all known histone acetyltransferase transcriptional co-activators (Haynes *et al.*, 1992; Tamkun *et al.*, 1992). This motif contains seven invariant residues, four of which are aromatic amino acids, and several conserved substitutions. Proteins containing multiple bromodomain can have the motifs either in tandem or separated by unrelated sequences (Haynes *et al.*, 1992). Solution structure of PCAF bromodomain (Dhalluin *et al.*, 1999) reveals an unusual left-handed, up-and-down four-helix bundle with a hydrophobic pocket able to interact with acetyl peptides or acetyl amino acids. The nature of the recognition of acetyl-lysine by the PCAF bromodomain is similar to that of acetyl-CoA by histone acetyltransferase, suggesting that bromodomain is functionally linked to the HAT activity of co-activators in the regulation of gene transcription. Structures of bromodomains of several other HATs substantiate this theory further (Hudson *et al.*, 2000; Jacobson *et al.*, 2000; Owen *et al.*, 2000; Mujtaba *et al.*, 2002, 2004). In the case of the double bromodomain (DBD) of human TAFII250, which is capable of binding multiple acetylated histones, the spacing between acetyl lysines (5/8 or 12/16 of histone H4) appears to be critical for determining the specificity of interaction (Jacobson *et al.*, 2000).

The importance of bromodomain-acetyl lysine association has been shown in several cases. The Bdf1 protein in yeast has two bromodomains that bind diacetylated H4 at high affinity (Matangkasombut and Buratowski, 2003). The acetylation-dependent interaction is important for determining the boundary between the transcriptionally active euchromatin and the silenced heterochromatin (Ladurner *et*

al., 2003). The tandem bromodomain of Rsc4 protein, part of the RSC chromatin remodelling complex, binds acetylated H3 and is important for transcription of a variety of yeast genes *in vivo* (Kasten *et al.*, 2004). Moreover, recruitment of the SWI/SNF chromatin remodelling complex has been shown to be facilitated by bromodomain-acetylated histone association (Syntichaki *et al.*, 2000; Hassan *et al.*, 2002).

CHROMODOMAIN

Like many other modifications on histones, context-dependent histone methylation provides a critical 'mark' on the chromatin, enabling the recruitment and binding of chromatin-associated proteins that direct specific biological response. The methyl lysine-binding, chromodomain-containing proteins play important roles in regulating gene activity and genome organization. The domain was first defined by Paro and Hogness (1991) as a conserved 37-residue region of homology present in *Drosophila* HP1 and Polycomb (Pc) proteins (Jones *et al.*, 2000; Brehm *et al.*, 2004). HP1, which binds to di/trimethylated H3-K9, is primarily associated with highly condensed and repressed chromatin (Jacobs *et al.*, 2001; Grewal and Elgin, 2002; Jacobs and Khorasanizadeh, 2002), while Polycomb works as an epigenetic repressor that regulates gene expression during development and binds trimethylated H3-K27 (Ringrose and Paro, 2001). The HP1-like chromodomain proteins (~25 kDa in size) (Singh *et al.*, 1991) share a conserved stretch of negatively charged amino acids adjacent to the N-terminus of the chromodomain, as well as an extensive C-terminal homology region called the chromo shadow domain. Pc-like proteins are larger in size and lack both these homology domains, but share a C-terminal homology called the Pc-box that is important for their function. In a positive feedback mode, HP1 bound to trimethylated H3-K9 recruits SUV39H1 that presumably methylates H3-K9 of the adjacent nucleosomes to promote further HP1 binding and resultant spread of heterochromatin (Nakayama *et al.*, 2001; Hall *et al.*, 2002). PC, on the other hand, is a component of the Polycomb repressive complex 1 (PRC1) that, once loaded onto the histone tails, blocks the access of the SWI/SNF chromatin remodelling complex or transcription initiation factors, thereby preventing decondensation of the chromatin by positive transcriptional regulators (Cao *et al.*, 2002; Czermin *et al.*, 2002; Kuzmichev *et al.*, 2002; Dellino *et al.*, 2004). H3-K9 and H3-K27 trimethylation is associated with the inactive X chromosome (Xi) (Heard *et al.*, 2001; Mermoud *et al.*, 2002; Plath *et al.*, 2003). Furthermore, H3-K9 methylation can trigger DNA methylation in *Neurospora crassa* (Tamaru and Selker, 2001) and *Arabidopsis thaliana* (Jackson *et al.*, 2002). The combination of histone- and DNA-methylation systems (Freitag and Selker, 2005) probably stabilizes silent chromatin domains, safeguarding gene expression programmes and protecting genome integrity.

The NMR structure of chomodomain from the mouse HP-like protein (HP1?) reveals a three-stranded, antiparallel β-sheet running across an α-helix, with the highly conserved residues of the domain forming a hydrophobic core (Ball *et al.*, 1997). Stable binding of HP1 to its target sites in the genome often requires multiple interactions besides histone H3 methylated at Lys9 (Cowieson *et al.*, 2000; Jacobs and Khorasanizadeh, 2002; Maison *et al.*, 2002; Nielsen *et al.*, 2002). The Polycomb chromodomain, however, does not distinguish a K9 methylated H3 tail from an

unmodified one *in vitro*. Rather, it shows increased affinity for methylated Lys27 of H3 brought about by the E(Z) methylase (Cao *et al.*, 2002; Czermin *et al.*, 2002).

Very similar to the chromodomain is the Tudor domain of the survival motor neuron SMN protein, which preferentially binds symmetrical dimethylarginine (Brahms *et al.*, 2001; Friesen *et al.*, 2001). The three-dimensional structure of the SMN Tudor domain is a strongly bent antiparallel β-sheet structure with a hydrophobic core constituted by the conserved residues (Selenko *et al.*, 2001). Structure of double tandem Tudor domains of 53BP1, a key transducer of the DNA damage checkpoint signal, reveals a new structural motif capable of binding both DNA and Arg–Gly-rich sequences (Charier *et al.*, 2004). The three β-stranded core region of Tudor and chromodomain is shared by PWWP, MBT, and Agenet domains, suggesting that these structurally related motifs may have evolved from a common ancestor and thus are grouped together as the Tudor domain 'Royal Family' (Maurer-Stroh *et al.*, 2003). On the other hand, it remains to be seen as to whether arginine-methylated histones are interaction targets for Tudor domain-containing proteins.

Are there more modified histone binding modules?

Methylation and acetylation only represent a portion of the repertoire of histone modifications. It is almost certain that other histone modifications also can act as the homing device to attract different proteins with specified molecular functions and activities to the underlying loci. This possibility is particularly attractive when opposite outcomes are linked to a single modification. For example, both transcriptional activation and chromatin condensation have been intimately linked to H3 Ser10 phosphorylation. Clearly, a unifying structural influence on chromatin behaviour cannot suffice the opposite requirement for these two nuclear activities. Cell cycle- or locus-dependent recruitment of selective factors that facilitate transcription or mitotic progression is a well-received hypothesis but with, as yet, little physical evidence. Furthermore, while many modules are known to bind protein modifications, among the most notable are 14-3-3 for phosphoserine/phosphothreonine (Yaffe, 2002a), and SH2 and PTB for phosphotyrosine (Yaffe, 2002b). The list of such modules is continually increasing. In fact, using a modified yeast two-hybrid approach, the identification of several yeast proteins that interact with acetylated histones H3 and H4 (Guo *et al.*, 2004) with diverse chromatin-related functions was recently reported. Intriguingly, none of these proteins contain the bromodomain. Similarly, a preliminary screen for yeast proteins that interact with phosphorylated carboxyl terminal domain (CTD) of the largest subunit of RNA polymerase II (encoded by *RPB1* in yeast) also uncovered several candidates without one of the canonical phosphoserine-interacting motifs (Guo *et al.*, 2004). These results underscore the necessity of non-biased screening, biochemically and genetically, for proteins that may be recruited by histones bearing specific post-translational modifications, and warrant the discovery of exciting new histone targets that rely on selective post-translational modifications.

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References

AASLAND, R., GIBSON, T.J. AND STEWART, A.F. (1995). The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends in Biochemical Sciences* **20**, 56–59.

ADAMS, R.R., CARMENA, M. AND EARNSHAW, W.C. (2001). Chromosomal passengers and the (Aurora) ABCs of mitosis. *Trends in Cell Biology* **11**, 49–54.

AHN, S.H., CHEUNG, W.L., HSU, J.Y., DIAZ, R.L., SMITH, M.M. AND ALLIS, C.D. (2005). Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in *S. cerevisiae*. *Cell* **120**, 25–36.

AKHTAR, A. AND BECKER, P.B. (2000). Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Molecular Cell* **5**, 367–375.

BALL, L.J., MURZINA, N.V., BROADHURST, R.W. *ET AL.* (1997). Structure of the chromatin binding (chromo) domain from mouse modifier protein 1. *EMBO Journal* **16**, 2473–2481.

BANNISTER, A.J., SCHNEIDER, R. AND KOUZARIDES, T. (2002). Histone methylation: dynamic or static? *Cell* **109**, 801–806.

BARRATT, M.J., HAZZALIN, C.A., CANO, E. AND MAHADEVAN, L.C. (1994). Mitogen-stimulated phosphorylation of histone H3 is targeted to a small hyperacetylation-sensitive fraction. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 4781–4785.

BAUER, U.M., DAUJAT, S., NIELSEN, S.J., NIGHTINGALE, K. AND KOUZARIDES, T. (2002). Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Reports* **3**, 39–44.

BAYER, P., ARNDT, A., METZGER, S. *ET AL.* (1998). Structure determination of the small ubiquitin-related modifier SUMO-1. *Journal of Molecular Biology* **280**, 275–286.

BIRCHLER, J.A., PAL-BHADRA, M. AND BHADRA, U. (2003). Dosage-dependent gene regulation and the compensation of the X chromosome in *Drosophila* males. *Genetica* **117**, 179–190.

BLANCO, J.C., MINUCCI, S., LU, J. *ET AL.* (1998). The histone acetylase PCAF is a nuclear receptor coactivator. *Genes and Development* **12**, 1638–1651.

BLANDER, G. AND GUARENTE, L. (2004). The Sir2 family of protein deacetylases. *Annual Reviews in Biochemistry* **73**, 417–435.

BODE, A.M. AND DONG, Z. (2003). Mitogen-activated protein kinase activation in UV-induced signal transduction. *Science's STKE* **167**, re2.

BODE, A.M. AND DONG, Z. (2005). Inducible covalent post-translational modification of histone H3. *Science's STKE* **281**, re4.

BRAHMS, H., MEHEUS, L., DE BRABANDERE, V., FISCHER, U. AND LUHRMANN, R. (2001). Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B' and the Sm-like protein LSm4, and their interaction with the SMN protein. *RNA* **7**, 1531–1542.

BREHM, A., TUFTELAND, K.R., ASHLAND, R. AND BECKER, P.B. (2004). The many colours of chromodomains. *Bioessays* **26**, 133–140.

BRIGGS, S.D., BRYK, M., STRAHL, B.D. *ET AL.* (2001). Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes and Development* **15**, 3286–3295.

BRIGGS, S.D., XIAO, T., SUN, Z.W. *ET AL.* (2002). Gene silencing: trans-histone regulatory pathway in chromatin. *Nature* **418**, 498.

BROWN, D.T. (2001). Histone variants: are they functionally heterogeneous? *Genome Biology* **2**, Review S0006.

BURMA, S., CHEN, B.P., MURPHY, M., KURIMASA, A. AND CHEN, D.J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *Journal of Biological Chemistry* **276**, 42462–42467.

BYLEBYL, G.R., BELICHENKO, I. AND JOHNSON, E.S. (2003). The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. *Journal of Biological Chemistry* **278**, 44113–44120.

BYVOET, P., SHEPHERD, G.R., HARDIN, J.M. AND NOLAND, B.J. (1972). The distribution and turnover of labelled methyl groups in histone fractions of cultured mammalian cells. *Archives of Biochemistry and Biophysics* **148**, 558–567.

CAIRNS, B.R. (2005). Chromatin remodelling complexes: strength in diversity, precision through specialization. *Current Opinion in Genetics and Development* **15**, 185–190.

CANDAU, R., ZHOU, J., ALLIS, C.D. AND BERGER, S.L. (1997). Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function *in vivo*. *EMBO Journal* **16**, 555–565.

CAO, R., WANG, L., WANG, H. *ET AL.* (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039–1043.

CARROZZA, M.J., UTLEY, R.T., WORKMAN, J.L. AND COTE, J. (2003). The diverse functions of histone acetyltransferase complexes. *Trends in Genetics* **19**, 321–329.

CHADEE, D.N., HENDZEL, M.J., TYLIPSKI, C.P. *ET AL.* (1999). Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. *Journal of Biological Chemistry* **274**, 24914–24920.

CHARIER, G., COUPRIE, J., ALPHA-BAZIN, B. *ET AL.* (2004). The Tudor tandem of 53BP1: a new structural motif involved in DNA and RG-rich peptide binding. *Structure* **12**, 1551–1562.

CHEN, D., MA, H., HONG, H. *ET AL.* (1999). Regulation of transcription by a protein methyltransferase. *Science* **284**, 2174–2177.

CHEN, H., LIN, R.J., SCHILTZ, R.L. *ET AL.* (1997). Nuclear receptor co-activator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with PCAF and CBP/p300. *Cell* **90**, 569–580.

CHEUNG, P., ALLIS, C.D. AND SASSONE-CORSI, P. (2000). Signalling to chromatin through histone modifications. *Cell* **103**, 263–271.

CHEUNG, W.L., AJIRO, K., SAMEJIMA, K. *ET AL.* (2003). Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* **113**, 507–517.

CHEUNG, W.L., TURNER, F.B., KRISHNAMOORTHY, T. *ET AL.* (2005). Phosphorylation of histone H4 serine 1 during DNA damage requires casein kinase II in *S. cerevisiae*. *Current Biology* **15**, 656–660.

CLARKE, A.S., LOWELL, J.E., JACOBSON, S.J. AND PILLUS, L. (1999). Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Molecular and Cellular Biology* **19**, 2515–2516.

CLAYTON, A.L. AND MAHADEVAN, L.C. (2003). MAP kinase-mediated phosphoacetylation of histone H3 and inducible gene regulation. *FEBS Letters* **546**, 51–58.

COWIESON, N.P., PARTRIDGE, J.F., ALLSHIRE, R.C. AND MCLAUGHLIN, P.J. (2000). Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Current Biology* **10**, 517–525.

CUTHBERT, G.L., DAUJAT, S., SNOWDEN, A.W. *ET AL.* (2004). Histone deimination antagonizes arginine methylation. *Cell* **118**, 545–553.

CZERMIN, B., MELFI, R., MCCABE, D., SEITZ, V., IMHOF, A. AND PIRROTTA, V. (2002). *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* **111**, 185–196.

DANIEL, J.A., TOROK, M.S., SUN, Z.W. *ET AL.* (2004). Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription. *Journal of Biological Chemistry* **279**, 1867–1871.

DELLINO, G.I., SCHWARTZ, Y.B., FARKAS, G., MCCABE, D., ELGIN, S.C. AND PIRROTTA, V. (2004). Polycomb silencing blocks transcription initiation. *Molecular Cell* **13**, 887–893.

DE NAPOLES, M., MERMOUD, J.E., WAKAO, R. *ET AL.* (2004). Polycomb group proteins ring 1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. *Developmental Cell* **7**, 663–676.

DENU, J. (2005). The Sir2 family of protein deacetylases. *Current Opinion in Chemical Biology* **9**, 431–440.

DHALLUIN, C., CARLSON, J.E., ZENG, L., HE, C., AGGARWAL, A.K. AND ZHOU, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496.

DI FIORE, P.P., POLO, S. AND HOFMANN, K. (2003). When ubiquitin meets ubiquitin receptors: a signalling connection. *Nature Reviews in Molecular and Cell Biology* **4**, 491–497.

DOU, Y., MIZZEN, C.A., ABRAMS, M., ALLIS, C.D. AND GOROVSKY, M.A. (1999). Phosphorylation of linker histone H1 regulates gene expression *in vivo* by mimicking H1 removal. *Molecular Cell* **4**, 641–647.

DOVER, J., SCHNEIDER, J., TAWIAH-BOATENG, M.A. *ET AL.* (2002). Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *Journal of Biological Chemistry* **277**, 28368–28371.

DOWNS, J.A., LOWNDES, N.F. AND JACKSON, S.P. (2000). A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408**, 1001–1004.

DOWNS, J.A., ALLARD, S., JOBIN-ROBITAILLE, O. *ET AL.* (2004). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Molecular Cell* **16**, 979–990.

DRUMMOND, D.C., NOBLE, C.O., KIRPOTIN, D.B., GUO, Z., SCOTT, G.K. AND BENZ, C.C. (2005). Clinical development of histone deacetylase inhibitors as anticancer agents. *Annual Review of Pharmacology and Toxicology* **45**, 495–528.

DUERRE, J.A. AND LEE, C.T. (1974). *In vivo* methylation and turnover of rat brain histones. *Journal of Neurochemistry* **23**, 541–547.

DUROCHER, D. AND JACKSON, S.P. (2001). DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme? *Current Opinion in Cell Biology* **13**, 225–231.

EBERHARTER, A. AND BECKER, P.B. (2002). Histone acetylation: a switch between repressive and permissive chromatin. *EMBO Reports* **3**, 224–229.

EHRENHOFER-MURRAY, A.E., RIVIER, D.H. AND RINE, J. (1997). The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. *Genetics* **145**, 923–934.

EMRE, N.C., INGVARSDOTTIR, K., WYCE, A. *ET AL.* (2005). Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. *Molecular Cell* **17**, 585–594.

FENG, Q., WANG, H., NG, H.H. *ET AL.* (2002). Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Current Biology* **12**, 1052–1058.

FOSTER, E.R. AND DOWNS, J.A. (2005). Histone H2A phosphorylation in DNA double-strand break repair. *FEBS Journal* **272**, 3231–3240.

FREITAG, M. AND SELKER, E.U. (2005). Controlling DNA methylation: many roads to one modification. *Current Opinion in Genetics and Development* **15**, 191–199.

FRIESEN, W.J., MASSENET, S., PAUSHKIN, S., WYCE, A. AND DREYFUSS, G. (2001). SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. *Molecular Cell* **7**, 1111–1117.

GARDNER, R.G., NELSON, Z.W. AND GOTTSCHLING, D.E. (2005). Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. *Molecular Cell Biology* **25**, 6123–6139.

GLOVER, D.M., LEIBOWITZ, M.H., MCLEAN, D.A. AND PARRY, H. (1995). Mutations in Aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* **81**, 95–105.

GOTO, H., TOMONO, Y., AJIRO, K. *ET AL.* (1999). Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. *Journal of Biological Chemistry* **274**, 25543–25549.

GOTO, H., YASUI, Y., NIGG, E.A. AND INAGAKI, M. (2002). Aurora-B phosphorylates histone H3 at serine28 with regard to the mitotic chromosome condensation. *Genes to Cells* **7**, 11–17.

GRANT, P.A., DUGGAN, L., COTE, J. *ET AL.* (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes and Development* **11**, 1640–1650.

GRANT, P.A., SCHIELTZ, D., PRAY-GRANT, M.G. *ET AL.* (1998). A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* **94**, 45–53.

GREWAL, S.I. AND ELGIN, S.C. (2002). Heterochromatin: new possibilities for the inheritance of structure. *Current Opinion in Genetics and Development* **12**, 178–187.

GROZINGER, C.M. AND SCHREIBER, S.L. (2002). Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chemistry and Biology* **9**, 3–16.

GRUNSTEIN, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349–352.

GUARENTE, L. (2005). Calorie restriction and SIR2 genes – towards a mechanism. *Mechanisms of Ageing and Development* **126**, 923–928.

GUO, D., HAZBUN, T.R., XU, X.J., NG, S.L., FIELDS, S. AND KUO, M.H. (2004). A tethered catalysis, two-hybrid system to identify protein–protein interactions requiring post-translational modifications. *Nature Biotechnology* **22**, 888–892.

HAKE, S.B., GARCIA, B.A., KAUER, M. *ET AL.* (2005). Serine 31 phosphorylation of histone variant H3.3 is specific to regions bordering centromeres in metaphase chromosomes. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 6344–6349.

HALL, I.M., SHANKARANARAYANA, G.D., NOMA, K., AYOUB, N., COHEN, A. AND GREWAL, S.I. (2002). Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232–2237.

HASSAN, A.H., PROCHASSON, P., NEELY, K.E. *ET AL.* (2002). Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell* **111**, 369–379.

HAY, R.T. (2005). SUMO: a history of modification. *Molecular Cell* **18**, 1–12.

HAYNES, S.R., DOLLARD, C., WINSTON, F., BECK, S., TROWSDALE, J. AND DAWID, I.B. (1992). The bromodomain: a conserved sequence found in human *Drosophila* and yeast proteins. *Nucleic Acids Research* **20**, 2603.

HEARD, E., ROUGEULLE, C., ARNAUD, D., AVNER, P., ALLIS, C.D. AND SPECTOR, D.L. (2001). Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. *Cell* **107**, 727–738.

HEBBES, T.R., THORNE, A.W. AND CRANE-ROBINSON, C. (1988). A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO Journal* **7**, 1395–1402.

HENDZEL, M.J., WEI, Y., MANCINI, M.A. *ET AL.* (1997). Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromasoma* **106**, 348–360.

HENIKOFF, S. AND AHMAD, K. (2005). Assembly of variant histones into chromatin. *Annual Reviews in Cell and Developmental Biology* **21**, 133–153.

HENRY, K.W. AND BERGER, S.L. (2002). Trans-tail histone modifications: wedge or bridge? *Nature Structural Biology* **9**, 565–566.

HENRY, K.W., WYCE, A., LO, W.S. *ET AL.* (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes and Development* **17**, 2648–2663.

HERSCHMAN, H.R. (1991). Primary response genes induced by growth factors and tumour promoters. *Annual Reviews in Biochemistry* **60**, 281–319.

HICKE, L. (2001). Protein regulation by monoubiquitin. *Nature Reviews in Molecular and Cell Biology* **2**, 195–201.

HOCHSTRASSER, M. (1996). Ubiquitin-dependent protein degradation. *Annual Reviews in Genetics* **30**, 405–439.

HSU, J.Y., SUN, Z.W., LI, X. *ET AL.* (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/Aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* **102**, 279–291.

HUDSON, B.P., MARTINEZ-YAMOUT, M.A., DYSON, H.J. AND WRIGHT, P.E. (2000). Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. *Journal of Molecular Biology* **304**, 355–370.

HWANG, W.W., VENKATASUBRAHMANYAM, S., IANCULESCU, A.G., TONG, A., BOONE, C. AND MADHANI, H.D. (2003). A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. *Molecular Cell* **11**, 261–266.

IYER, N.G., OZDAG, H. AND CALDAS, C. (2004). p300/CBP and cancer. *Oncogene* **23**, 4225–4231.

JACKSON, J.P., LINDROTH, A.M., CAO, X. AND JACOBSEN, S.E. (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560.

JACOBS, S.A. AND KHORASANIZADEH, S. (2002). Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* **295**, 2080–2083.

JACOBS, S.A., TAVERNA, S.D., ZHANG Y. *ET AL.* (2001). Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO Journal* **20**, 5232–5234.

JACOBSON, R.H., LADURNER, A.G., KING, D.S. AND TJIAN, R. (2000). Structure and function of a human TAFII250 double bromodomain module. *Science* **288**, 1422–1425.

JASON, L.J., MOORE, S.C., LEWIS, J.D., LINDSEY, G. AND AUSIO, J. (2002). Histone ubiquitination: a tagging tail unfolds? *Bioassays* **24**, 166–174.

JEPSEN, K. AND ROSENFELD, M.G. (2002). Biological roles and mechanistic actions of co-repressor complexes. *Journal of Cell Science* **115**, 689–698.

JONES, D.O., COWELL, I.G. AND SINGH, P.B. (2000). Mammalian chromodomain proteins: their role in genome organisation and expression. *Bioessays* **22**, 124–137.

KALKHOVEN, E. (2004). CBP and p300: HATs for different occasions. *Biochemical Pharmacology* **68**, 1145–1155.

KAMAKAKA, R.T. (2003). Heterochromatin: proteins in flux lead to stable repression. *Current Biology* **13**, R317–319.

KAMAKAKA, R.T. AND BIGGINS, S. (2005). Histone variants: deviants? *Genes and Development* **19**, 295–310.

KASTEN, M., SZERLONG, H., ERDJUMENT-BROMAGE, H., TEMPST, P., WERNER, M. AND CAIRNS, B.R. (2004). Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. *EMBO Journal* **23**, 1348–1359.

KHORASANIZADEH, S. (2004). The nucleosome: from genomic organization to genomic regulation. *Cell* **116**, 259–272.

KOUZARIDES, T. (2002). Histone methylation in transcriptional control. *Current Opinion in Genetics and Development* **12**, 198–209.

KROGAN, N.J., DOVER, J., WOOD, A. *ET AL.* (2003). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Molecular Cell* **11**, 721–729.

KUNDU, T.K., PALHAN, V.B., WANG, Z., AN, W., COLE, P.A. AND ROEDER, R.G. (2000). Activator-dependent transcription from chromatin *in vitro* involving targeted histone acetylation by p300. *Molecular Cell* **6**, 551–561.

KUO, M.H., BROWNELL, J.E., SOBEL, R.E. *ET AL.* (1996). Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383**, 269–272.

KUO, M.-H., ZHOU, J., JAMBECK, P., CHURCHILL, M.E.A. AND ALLIS, C.D. (1998). Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes *in vivo*. *Genes and Development* **12**, 627–639.

KUSCH, T., FLORENS, L., MACDONALD, W.H. *ET AL.* (2004). Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* **306**, 2084–2087.

KUZMICHEV, A., NISHIOKA, K., ERDJUMENT-BROMAGE, H., TEMPST, P. AND REINBERG, D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes and Development* **16**, 2893–2905.

LACHNER, M. AND JENUWEIN, T. (2002). The many faces of histone lysine methylation. *Current Opinion in Cell Biology* **14**, 286–298.

LACOSTE, N., UTLEY, R.T., HUNTER, J.M., POIRIER, G.G. AND COTE, J. (2002). Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. *Journal Biological Chemistry* **277**, 30421–30424.

LADURNER, A.G., INOUYE, C., JAIN, R. AND TJIAN, R. (2003). Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Molecular Cell* **11**, 365–376.

LEE, J.H., COOK, J.R., POLLACK, B.P., KINZY, T.G., NORRIS, D. AND PESTKA, S. (2000). Hsl7p, the yeast homologue of human JBP1, is a protein methyltransferase. *Biochemical and Biophysical Research Communications* **274**, 105–111.

LEE, M.G., WYNDER, C., COOCH, N. AND SHIEKHATTAR, R. (2005). An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* **437**, 432–435.

LEHEMBRE, F., BADENHORST, P., MÜLLER, S., TRAVERS, A., SCHWEISGUTH, F. AND DEJEAN, A. (2000). Covalent modification of the transcriptional repressor tramtrack by the ubiquitin-related protein smt3 in *Drosophila* flies. *Molecular and Cellular Biology* **20**, 1072–1082.

LEO, C. AND CHEN, J.D. (2000). The SRC family of nuclear receptor coactivators. *Gene* **245**, 1–11.

LI, Y., WANG, H., WANG, S., QUON, D., LIU, Y.-H. AND CORDELL, B. (2003). Positive and negative regulation of APP amyloidogenesis by sumoylation. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 259–264.

LO, W.S., DUGGAN, L., EMRE, N.C. *ET AL.* (2001). Snf1 – a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. *Science* **293**, 1142–1146.

LO, W.S., GAMACHE, E.R., HENRY, K.W., YANG, D., PILLUS, L. AND BERGER, S.L. (2005). Histone H3 phosphorylation can promote TBP recruitment through distinct promoter-specific mechanisms. *EMBO Journal* **24**, 997–1008.

LUCCHESI, J.C. (1998). Dosage compensation in flies and worms: the ups and downs of X-chromosome regulation. *Current Opinion in Genetics and Development* **8**, 179–184.

LUGER, K. (2003). Structure and dynamic behaviour of nucleosomes. *Current Opinion in Genetics and Devlopment* **13**, 127–135.

LUNYAK, V.V., BURGESS, R., PREFONTAINE, G.G. *ET AL.* (2002). Co-repressor-dependent silencing of chromosomal regions encoding neuronal genes. *Science* **298**, 1747–1752.

MA, H., BAUMANN, C.T., LI, H. *ET AL.* (2001). Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. *Current Biology* **11**, 1981–1985.

MADIGAN, J.P., CHOTKOWSKI, H.L. AND GLASER, R.L. (2002). DNA double-strand break-induced phosphorylation of *Drosophila* histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Research* **30**, 3698–3705.

MAHADEVAN, L.C., WILLIS, A.C. AND BARRATT, M.J. (1991). Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* **65**, 775–783.

MAISON, C., BAILLY, D., PETERS, A.H. *ET AL.* (2002). Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nature Genetics* **30**, 329–334.

MALIK, H.S. AND HENIKOFF S. (2003). Phylogenomics of the nucleosome. *Nature Structural Biology* **10**, 882–891.

MARMORSTEIN, M. (2004). Structure and chemistry of the Sir2 family of NAD+-dependent histone/protein deactylases. *Biochemical Society Transactions* **32**, 904–909.

MARMORSTEIN, R. (2001). Structure of histone deacetylases: insights into substrate recognition and catalysis. *Structure (Camb)* **9**, 1127–1133.

MARMORSTEIN, R. AND ROTH, S.Y. (2001). Histone acetyltransferases: function, structure and catalysis. *Current Opinion in Genetics and Development* **11**, 155–161.

MARTÍNEZ-BALBÁS, M.A., BANNISTER, A.J., MARTIN, K., HAUS-SEUFFERT, P., MEISTERERNST, M. AND KOUZARIDES, T. (1998). The acetyltransferase activity of CBP stimulates transcription. *EMBO Journal* **17**, 2886–2893.

MATANGKASOMBUT, O. AND BURATOWSKI, S. (2003). Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. *Molecular Cell* **11**, 353–363.

MAURER-STROH, S., DICKENS, N.J., HUGHES-DAVIES, L., KOUZARIDES, T., EISENHABER, F. AND PONTING, C.P. (2003). The Tudor domain 'Royal Family': Tudor, plant Agenet, Chromo, PWWP and MBT domains. *Trends in Biochemical Sciences* **28**, 69–74.

MCKITTRICK, E., GAFKEN, P.R., AHMAD, K. AND HENIKOFF, S. (2004). Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 1525–1530.

MELUH, P.B., YANG, P., GLOWCZEWSKI, L., KOSHLAND, D. AND SMITH, M.M. (1998). Cse4p is a component of the core centromere of *Saccharomyces cerevisiae*. *Cell* **94**, 607–613.

MERMOUD, J.E., POPOVA, B., PETERS, A.H., JENUWEIN, T. AND BROCKDORFF, N. (2002). Histone H3 lysine 9 methylation occurs rapidly at the onset of random X chromosome inactivation. *Current Biology* **12**, 247–251.

METZGER, E., WISSMANN, M., YIN, N. *ET AL.* (2005). LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **437**, 436–439.

MORRISON, A.J., HIGHLAND, J., KROGAN, N.J. *ET AL.* (2004). INO80 and γ-H2AX interaction links ATP-dependent chromatin remodelling to DNA damage repair. *Cell* **119**, 767–775.

MUELLER, C.L., PORTER, S.E., HOFFMAN, M.G. AND JAEHNING, J.A. (2004). The Paf1 complex has functions independent of actively transcribing RNA polymerase II. *Molecular Cell* **14**, 447–456.

MUJTABA, S., HE, Y., ZENG, L. *ET AL.* (2002). Structural basis of lysine-acetylated HIV-1 Tat recognition by PCAF bromodomain. *Molecular Cell* **9**, 575–586.

MUJTABA, S., HE, Y., ZENG, L. *ET AL.* (2004). Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. *Molecular Cell* **13**, 251–263.

MURRAY, K. (1964). The occurrence of epsilon-N-methyl lysine in histones. *Biochemistry* **127**, 10–15.

NAKAMURA, T.M.L.-L., REDON, C. AND RUSSELL, P. (2004). Histone H2A phosphorylation controls Crb2 recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast. *Molecular and Cellular Biology* **24**, 6215–6230.

NAKAYAMA, J., RICE, J.C., STRAHL, B.D., ALLIS, C.D. AND GREWAL, S.I. (2001). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110–113.

NARLIKAR, G.J., FAN, H.Y. AND KINGSTON, R.E. (2002). Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487.

NG, H.H., XU, R.M., ZHANG, Y. AND STRUHL, K. (2002a). Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *Journal of Biological Chemistry* **277**, 34655–34657.

NG, H.H., FENG, Q., WANG, H. *ET AL.* (2002b). Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes and Development* **16**, 1518–1527.

NG, H.H., DOLE, S. AND STRUHL, K. (2003). The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *Journal of Biological Chemistry* **278**, 33625–33628.

NIELSEN, P.R., NIETLISPACH, D., MOTT, H.R. *ET AL.* (2002). Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature* **416**, 103–107.

NIELSEN, S.J., SCHNEIDER, R., BAUER, U.M. *ET AL.* (2001). Rb targets histone H3 methylation and HP1 to promoters. *Nature* **412**, 561–565.

NISHIOKA, K., RICE, J.C., SARMA, K. *ET AL.* (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Molecular Cell* **9**, 1201–1213.

NOWAK, S.J. AND CORCES, V.G. (2004). Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends in Genetics* **20**, 214–220.

OGAWA, H., ISHIGURO, K., GAUBATZ, S., LIVINGSTON, D.M. AND NAKATANI, Y. (2002). A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* **296**, 1132–1136.

OGRYZKO, V.V., SCHILTZ, R.L., RUSSANOVA, V., HOWARD, B.H. AND NAKATANI, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**, 953–959.

OGRYZKO, V.V., KOTANI, T., ZHANG, X. *ET AL.* (1998). Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94**, 35–44.

OWEN, D.J., ORNAGHI, P., YANG, J.C. *ET AL.* (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO Journal* **19**, 6141–6149.

PALMER, D.K., O'DAY, K., TRONG, H.L., CHARBONNEAU, H. AND MARGOLIS, R.L. (1991). Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 3734–3738.

PARO, R. AND HOGNESS, D.S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 263–267.

PARTHUN, M.R., WIDOM, J. AND GOTTSCHLING, D.E. (1996). The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell* **87**, 85–94.

PETERSEN, J., PARIS, J., WILLER, M., PHILIPPE, M. AND HAGAN, I.M. (2001). The *S. pombe* Aurora-related kinase Ark1 associates with mitotic structures in a stage dependent manner and is required for chromosome segregation. *Journal of Cell Science* **114**, 4371–4384.

PETRIJ, F., GILES, R.H., DAUWERSE, H.G. *ET AL.* (1995). Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* **376**, 348–351.

PICKART, C.M. (2001). Mechanisms underlying ubiquitination. *Annual Reviews in Biochemistry* **70**, 503–533.

PICKART, C.M. (2004). Back to the future with ubiquitin. *Cell* **116**, 181–190.

PINA, B. AND SUAU, P. (1987). Changes in histone H2A and H3 variant composition in differentiating and mature rat brain cortical neurons. *Developmental Biology* **123**, 51–58.

PLATH, K., FANG, J., MLYNARCZYK-EVANS, S.K. *ET AL.* (2003). Role of histone H3 lysine 27 methylation in X inactivation. *Science* **300**, 131–135.

POLIOUDAKI, H., MARKAKI, Y., KOURMOULI, N. *ET AL.* (2004). Mitotic phosphorylation of histone H3 at threonine 3. *FEBS Letters* **560**, 39–44.

PREUSS, U., LANDSBERG, G. AND SCHEIDTMANN, K.H. (2003). Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase. *Nucleic Acids Research* **31**, 878–885.

PRIGENT, C. AND DIMITROV, S. (2003). Phosphorylation of serine 10 in histone H3, what for? *Journal of Cell Science* **116**, 3677–3685.

PRIGENT, C. AND GIET, R. (2003). Aurora A and mitotic commitment. *Cell* **114**, 531–532.

PURI, P.L., SARTORELLI, V., YANG, X.J. *ET AL.* (1997). Differential roles of p300 and PCAF acetyltransferases in muscle differentiation. *Molecular Cell* **1**, 35–45.

REA, S., EISENHABER, F., O'CARROLL, D. *ET AL.* (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599.

REDON, C., PILCH, D.R., ROGAKOU, E., ORR, A.H., LOWNDES, N.F. AND BONNER, W.M. (2003). Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. *EMBO Reports* **4**, 678–684.

REIFSNYDER, C., LOWELL, J., CLARKE, A. AND PILLUS, L. (1996). Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nature Genetics* **14**, 42–49.

REN, Q. AND GOROVSKY, M.A. (2001). Histone H2A.Z acetylation modulates an essential charge patch. *Molecular Cell* **7**, 1329–1335.

RICE, J.C., BRIGGS, S.D., UEBERHEIDE, B. *ET AL.* (2003). Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Molecular Cell* **12**, 1591–1598.

RINGROSE, L. AND PARO, R. (2001). Remembering silence. *Bioessays* **23**, 566–570.

ROBYR, D., SUKA, Y., XENARIOS, I. *ET AL.* (2002). Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell* **109**, 437–446.

ROBZYK, K., RECHT, J. AND OSLEY, M.A. (2000). Rad6-dependent ubiquitination of histone H2B in yeast. *Science* **287**, 501–504.

ROGAKOU, E.P., PILCH, D.R., ORR, A.H., IVANOVA, V.S. AND BONNER, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *Journal of Biological Chemistry* **273**, 5858–5868.

ROTH, S.Y., DENU, J.M. AND ALLIS, C.D. (2001). Histone acetyltransferases. *Annual Reviews in Biochemistry* **70**, 81–120.

RUIZ-GARCÍA, A.B., SENDRA, R., GALIANA, M., PAMBLANCO, M., PEREZ-ORTIN, J.E. AND TORDERA, V. (1998). HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. *Journal of Biological Chemistry* **273**, 12599–12605.

SALVADOR, L.M., PARK, Y., COTTOM, J. *ET AL.* (2001). Follicle-stimulating hormone stimulates protein kinase A-mediated histone H3 phosphorylation and acetylation leading to select gene activation in ovarian granulosa cells. *Journal of Biological Chemistry* **276**, 40146–40155.

SANTOS-ROSA, H., SCHNEIDER, R., BANNISTER, A.J. *ET AL.* (2002). Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411.

SASSONE-CORSI, P., MIZZEN, C.A., CHEUNG, P. *ET AL.* (1999). Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. *Science* **285**, 886–891.

SCHNELL, J.D. AND HICKE, L. (2003). Non-traditional functions of ubiquitin and ubiquitin-binding proteins. *Journal of Biological Chemistry* **278**, 35857–35860.

SCHUMACHER, J.M., GOLDEN, A. AND DONOVAN, P.J. (1998). AIR-2: an Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. *Journal of Cell Biology* **143**, 1635–1646.

SEELER, J.S. AND DEJEAN, A. (2003). Nuclear and unclear functions of SUMO. *Nature Reviews in Molecular and Cell Biology* **4**, 690–699.

SELENKO, P., SPRANGERS, R., STIER, G., BUHLER, D., FISCHER, U. AND SATTLER, M. (2001). SMN tudor domain structure and its interaction with the Sm proteins. *Nature Structure Biology* **8**, 27–31.

SHI, Y., LAN, F., MATSON, C. *ET AL.* (2004). Histone demethylation mediated by the nuclear amine oxidase homologue LSD1. *Cell* **119**, 941–953.

SHIBATA, K., INAGAKI, M. AND AJIRO, K. (1990). Mitosis-specific histone H3 phosphorylation *in vitro* in nucleosome structure. *European Journal of Biochemistry* **193**, 87–93.

SHIIO, Y. AND EISENMAN, R.N. (2003). Histone sumoylation is associated with transcriptional repression. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 13225–13230.

SHOEMAKER, C.B. AND CHALKLEY, R. (1980). H3-specific nucleohistone kinase of bovine thymus chromatin. Purification, characterization, and specificity for threonine residue 3. *Journal of Biological Chemistry* **255**, 11048–11055.

SHROFF, R., ARBEL-EDEN, A., PILCH, D. *ET AL.* (2004). Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Current Biology* **14**, 1703–1711.

SIMS, R.J., 3RD, NISHIOKA, K. AND REINBERG, D. (2003). Histone lysine methylation: a signature for chromatin function. *Trends in Genetics* **19**, 629–639.

SINGH, P.B., MILLER, J.R., PEARCE, J. *ET AL.* (1991). A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants. *Nucleic Acids Research* **19**, 789–794.

SPELIOTES, E.K., UREN, A., VAUX, D. AND HORVITZ, H.R. (2000). The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Molecular Cell* **6**, 211–223.

SPENCE, J., GALI, R.R., DITTMAR, G., SHERMAN, F., KARIN, M. AND FINLEY, D. (2000). Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain. *Cell* **102**, 67–76.

STALLCUP, M.R. (2001). Role of protein methylation in chromatin remodelling and transcriptional regulation. *Oncogene* **20**, 3014–3020.

STEWART, G.S., WANG, B., BIGNELL, C.R., TAYLOR, A.M. AND ELLEDGE, S.J. (2003). MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* **421**, 961–966.

STIFF, T., O'DRISCOLL, M., RIEF, N., IWABUCHI, K., LOBRICH, M. AND JEGGO, P.A. (2004). ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Research* **64**, 2390–2396.

STRAHL, B.D., OHBA, R., COOK, R.G. AND ALLIS, C.D. (1999). Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 14967–14972.

STRAHL, B.D., BRIGGS, S.D., BRAME, C.J. *ET AL.* (2001). Methylation of histone H4 at arginine 3 occurs *in vivo* and is mediated by the nuclear receptor coactivator PRMT1. *Current Biology* **11**, 996–1000.

STRUHL, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. *Genes and Development* **12**, 599–606.

SUGIYAMA, K., SUGIURA, K., HARA, T. *ET AL.* (2002). Aurora-B associated protein phosphatases as negative regulators of kinase activation. *Oncogene* **21**, 3103–3111.

SUN, Z.W. AND ALLIS, C.D. (2002). Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* **418**, 104–108.

SYNTICHAKI, P., TOPALIDOU, I. AND THIREOS, G. (2000). The Gcn5 bromodomain co-ordinates nucleosome remodelling. *Nature* **404**, 414–417.

TACHIBANA, M., SUGIMOTO, K., FUKUSHIMA, T. AND SHINKAI, Y. (2001). Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *Journal of Biological Chemistry* **276**, 25309–25317.

TAMARU, H. AND SELKER, E.U. (2001). A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* **414**, 277–283.

TAMKUN, J.W., DEURING, R., SCOTT, M.P. *ET AL.* (1992). Brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**, 561–572.

THORNE, A.W., SAUTIERE, P., BRIAND, G. AND CRANE-ROBINSON, C. (1987). The structure of ubiquitinated histone H2B. *EMBO Journal* **6**, 1005–1010.

TIMMERMANN, S., LEHRMANN, H., POLESSKAYA, A. AND HAREL-BELLAN, A. (2001). Histone acetylation and disease. *Cell and Molecular Life Sciences* **58**, 728–736.

TREWICK, S.C., MCLAUGHLIN, P.J. AND ALLISHIRE, R.C. (2005). Methylation: lost in hydroxylation? *EMBO Reports* **6**, 315–320.

UNAL, E., ARBEL-EDEN, A., SATTLER, U. *ET AL.* (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Molecular Cell* **16**, 991–1002.

VAN ATTIKUM, H., FRITSCH, O., HOHN, B. AND GASSER, S.M. (2004). Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodelling with DNA double-strand break repair. *Cell* **119**, 777–788.

VAN LEEUWEN, F., GAFKEN, P.R. AND GOTTSCHLING, D.E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109**, 745–756.

WANG, H., CAO, R., XIA, L. *ET AL.* (2001a). Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Molecular Cell* **8**, 1207–1217.

WANG, H., HUANG, Z.Q., XIA, L. *ET AL.* (2001b). Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* **293**, 853–857.

WANG, H., WANG, L., ERDJUMENT-BROMAGE, H. *ET AL.* (2004b). Role of histone H2A ubiquitination in Polycomb silencing. *Nature* **431**, 873–878.

WANG, L., LIU, L. AND BERGER, S.L. (1998). Critical residues for histone acetylation by GCN5, functioning in Ada and SAGA complexes, are also required for transcriptional function *in vivo*. *Genes and Development* **12**, 640–653.

WANG, Y., WYSOCKA, J., SAYEGH, J. *ET AL.* (2004a). Human PAD4 regulates histone arginine methylation levels via demethylation. *Science* **306**, 279–283.

WARD, I.M., MINN, K., JORDA, K.G. AND CHEN, J. (2003). Accumulation of checkpoint protein 53BP1 at DNA breaks involves its binding to phosphorylated histone H2AX. *Journal of Biological Chemistry* **278**, 19579–19582.

WEI, Y., MIZZEN, C.A., COOK, R.G., GOROVSKY, M.A. AND ALLIS, C.D. (1998). Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in *Tetrahymena*. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 7480–7484.

WEI, Y., YU, L., BOWEN, J., GOROVSKY, M.A. AND ALLIS, C.D. (1999). Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* **97**, 99–109.

WITTSCHIEBEN, B.Ø., OTERO, G., DE BIZEMONT, T. *ET AL.* (1999). A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Molecular Cell* **4**, 123–128.

WOLFFE, A.P. (2001). Transcriptional regulation in the context of chromatin structure. *Essays in Biochemistry* **37**, 45–57.

WOOD, A., KROGAN, N.J., DOVER, J. *ET AL.* (2003a). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Molecular Cell* **11**, 267–274.

WOOD, A., SCHNEIDER, J., DOVER, J., JOHNSTON, M. AND SHILATIFARD, A. (2003b). The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *Journal of Biological Chemistry* **278**, 34739–34742.

XU, L., LAVINSKY, R.M., DASEN, J.S. *ET AL.* (1998a). Signal-specific co-activator domain requirements for Pit-1 activation. *Nature* **395**, 301–306.

XU, W., EDMONDSON, D.G. AND ROTH, S.Y. (1998b). Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. *Molecular and Cellular Biology* **18**, 5659–5669.

YAFFE, M.B. (2002a). How do 14-3-3 protein work? – Gatekeeper phosphorylation and the molecular anvil hypothesis. *FEBS Letters* **513**, 53–57.

YAFFE, M.B. (2002b). Phosphotyrosine-binding domains in signal transduction. *Nature Reviews Molecular Cell Biology* **3**, 177–186.

YAMAMOTO, Y., VERMA, U.N., PRAJAPATI, S., KWAK, Y.T. AND GAYNOR, R.B. (2003). Histone H3 phosphorylation by IKK-alpha is critical for cytokine-induced gene expression. *Nature* **423**, 655–659.

ZHANG, K.L., LIN, W., LATHAM, J.A. *ET AL.* (2005). The Set1 methyltransferase opposes Ipl1 Aurora kinase functions in chromosome segregation. *Cell* **122**, 723–734.

ZHANG, Y. AND REINBERG, D. (2001). Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes and Development* **15**, 2343–2360.

ZHONG, S.P., MA, W.Y. AND DONG, Z. (2000). ERKs and p38 kinases mediate ultraviolet B-induced phosphorylation of histone H3 at serine 10. *Journal of Biological Chemistry* **275**, 20980–20984.

ZHONG, S., JANSEN, C., SHE, Q.-B. *ET AL.* (2001). Ultraviolet B-induced phosphorylation of histone H3 at serine 28 is mediated by MSK1. *Journal of Biological Chemistry* **276**, 33213–33219.

ZHONG, S., GOTO, H., INAGAKI, M. AND DONG, Z. (2003). Phosphorylation at serine 28 and acetylation at lysine 9 of histone H3 induced by trichostatin A. *Oncogene* **22**, 5291–5297.