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Open Chromatin

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ABSTRACT

In eukaryotic cells genomic DNA is packaged into chromatin thereby constraining the size of the molecule and allowing the cell to organise the genetic information within the nucleus. However, due to this enormous compaction, the accessibility of particular genomic regions that are required for several fundamental cellular processes such as transcription, replication, DNA repair and recombination is impaired. The reversion of this "locked" state, preparatory to and in the course of such processes, involves numerous epigenetic adaptations that finally embody an open chromatin conformation. These alterations comprise the post-translational modifications of histones as well as the activity of chromatin remodelling complexes and the incorporation of particular non-canonical histone variants although these mechanisms may work as one entity rather than single separated mechanisms. Rapid and transient alterations in the local chromatin structure are distinct from more stable marks that may comprise an epigenetic memory, as they rapidly respond to cellular demands rather than stipulate a particular function. Nevertheless, evidence for an interdependency of histone post-translational modifications, like acetylation, methylation, phosphorylation, ADP-ribosylation and sumoylation, and a role of such "marks" in regulating the accessibility of chromatin, supports the model of a histone code. This hypothesis extends the genetic information by an epigenetic component and regulates the interaction of chromatin with detector proteins. In this review we discuss how the chromatin status is adapted to environmental cues and which factors are involved in the formation and interpretation of open chromatin.

Keywords: chromatin remodelling, epigenetics, euchromatin, heterochromatin, histone code, histone modifications, histone variants, modification crosstalk, signal transduction, transcription

Abbreviations: FAD, flavin adenine dinucleotide; **GR**, glucocorticoid receptor; **HAT**, histone acetyltransferase; **HDAC**, histone deacetylase; **HDI**, histone deacetylase inhibitor; **HMTase**, histone (lysine) methyltransferase; **HP1**, heterochromatin protein 1; **HRE**, hormone response elements; **IE**, immediate early; **MAP-kinase**, mitogen activated protein kinase; **MBD**, methyl binding domain; **MMTV**, mouse mammary tumour virus; **PR**, progesterone receptor; **PTM**, post-translational modification; **RNAP II**, RNA polymerase II

CONTENTS

INTRODUCTION

The cellular response to environmental cues such as cellular stress, change of metabolic conditions, or differentiation signals is mediated via the activation of signal transduction cascades (reviewed in Downward (2001)). Stimulation of such pathways entails an alteration and adaptation of the gene expression profile to equip the cell for a proper response to novel and sometimes menacing demands. During the last decades numerous cellular signal transduction path-

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Invited Review

ways and molecules involved have been identified and characterised, expanding our knowledge on how the cell recognises signals and relays them. To access the biological information embedded in the genome, genes have to be transcribed in the context of a debilitating chromatin environment and signal transduction frequently affects the chromatin level.

Therefore, to initiate and conduct transcription the chromatin polymer is subjected to dynamic structural alterations that finally confer the formation of a transcriptional competent environment. Here we discuss three different mechanisms, the post-translational modification (PTM) of histones, chromatin remodelling via ATP-dependent machineries and the incorporation of particular histone-variants that lead to a preparation and adaptation of the chromatin environment to transcription.

HISTONE POST-TRANSLATIONAL MODIFICATIONS AND MODIFICATION CROSSTALK

The first observations that acetylation and methylation of histone molecules impact RNA synthesis emphasized the role of these molecules and their PTMs in the process of transcription (Allfrey *et al*. 1964). With the establishment of histone acetyltransferases (HATs) as transcriptional co-activators (Brownell *et al*. 1996; Mizzen *et al*. 1996) and histone deacetylases (HDAC) as co-repressors (Taunton *et al*. 1996), post-translational histone modifications were ultimately stated as a pivotal point in the modulation of genome accessibility. The repertoire of known histone PTMs has since been considerably expanded and modifications like lysine or arginine methylation, lysine acetylation, ADP-ribosylation and ubiquitination or serine and threonine phosphorylation have been correlated with transcriptional active or repressive chromatin states. The plethora of possible histone modification patterns epitomises the enormous potential information content that may expand the genetic information and defines local and global chromatin states via an additional level termed the histone code (Strahl and Allis 2000; Jenuwein and Allis 2001).

Epigenetic crosstalk: Stable versus dynamic modifications

The term epigenetic defines heritable changes in gene expression that do not involve changes of the DNA sequence. Such epigenetic traits are established during differentiation and comprehend DNA methylation, RNA-induced silencing and histone PTMs. These marks are essential for defining and maintaining cellular identity (Turner 2002; Egger *et al*. 2004), which is also emphasized by the observation that tumour cells display far-reaching epigenetic alterations during transformation (Fraga and Esteller 2005).

Histone PTMs are now recognised as important mediators of epigenetic traits that are crucial for defining specialised chromatin states like constitutive or facultative heterochromatin and the establishment and maintenance of cell-type specific gene expression profiles. There is accumulating evidence that different histone PTMs occur as interdependent events and frequently one initial modification may stipulate the context for subsequent events. The basis for such modification based crosstalk may originate either (1) from the assembly of multi-protein complexes that amalgamate several distinct chromatin modifying activities, (2) by supporting or hampering of protein-protein interactions or (3) via a direct influence of combinatorial PTMs on enzymatic activities. Therefore, the modification "make-up" of a histone molecule prior to a particular stimulus may provide the context for consecutive modification events. In addition, stimulus induced PTMs may provide the basis for subsequent "delayed"-modifications and may themselves be biased by local pre-modifications. Such PTM directed intra-molecular signalling is not limited to histone modifications but rather appears as an important mechanism in the regulation of various cellular processes (Yang 2005). These considerations are even meaningful for the definition of epigenetic marks: modifications with high turnover rates such as lysine acetylation are often considered as irrelevant for epigenetic mechanisms due to their dynamic turnover and ephemerality. Given the interdependency and sequential placement of PTMs, even rapidly processed modifications could epitomise a particular epigenetic status possibly dependent on other more stable marks, but with reciprocal connection. Since lysine acetylation is in general associated with transcriptional active regions, the local underrepresentation of this modification (hypoacetylation) could be interpreted as transcriptional silent hallmark, constituting an epigenetic state dependent on a previously established repressive environment via more stable histone modifications (secondary epigenetic marks). Such repressive heterochromatic states are established during differentiation via relatively stable marks such as CpG island methylation and methylation of histone H3 at K9 and K27 in conjunction with heterochromatin protein1 (HP1) or polycomb-group proteins (PcG), respectively (Lachner *et al*. 2003). The view of K9 methylation and HP1 recruitment as an exclusively repressive hallmark has been questioned by reports on the localisation of both factors to transcribed regions and reports on a H3K9me3 independent recruitment of HP1β (Vakoc *et al*. 2005; Dialynas *et al*. 2006). Methylation marks at K9 and K27 of histone H3 have a relatively long half-life and were therefore considered to be real (primary) epigenetic marks. However, recent studies showed that histone methylation is dynamically regulated by histone methyltransferases (HMTases) and newly identified demethylases (reviewed in Shi and Whetstine 2006). Two different types of enzymes have been found to act as histone demethylases up to now. Lysine-specific demethylase 1 (LSD1), a family member of the flavin adenine dinucleotide (FAD) dependent amine oxidases has been originally identified as component of co-repressor complexes (Tong *et al*. 1998; Humphrey *et al*. 2001) and was lately shown to demethylate H3K4me1/2 (Shi *et al*. 2004). In the presence of the androgen receptor LSD1 can also function as H3K9 demethylase thereby acting as transcriptional activator (Metzger *et al*. 2005). Jumonji C (JmjC) domain containing proteins, which are dioxygenases, were the second group of enzymes shown to be associated with histone demethylase activity (Trewick *et al*. 2005). JHDM2 (JmjC domain-containing histone demethylase 2) was demonstrated to specifically demethylate H3K9me2 (Yamane *et al*. 2006). Trimethylation of lysine 9, and also of lysine 36, is reversed by another recently identified histone demethylase subfamily, the so-called JMJD2 that also contains a JmjC domain (Whetstine *et al*. 2006). Surprisingly, JMJD2A was previously identified as a transcriptional repressor (Gray *et al*. 2005; Zhang *et al*. 2005), underlining its dual function by demethylating K9, a repressive mark, but also K36, an active mark.

Up to now, no specific K27 demethylase was reported. Methylated K27 constitutes a binding epitope for polycomb group proteins, which is needed to maintain the silenced state of homeotic genes, like the *hox* genes, and is additionally required for X chromosome inactivation (Wang *et al*. 2001).

In contrast to methylation, acetylation of histones comprises a modification with much more rapid turnover rates, and transcriptional active regions are in general enriched for acetylated nucleosomes (hyperacetylation) whereas transcriptional suppressed areas are mainly hypoacetylated. Studies on the genome wide distribution of histone acetylation suggest a two-phased distribution of this modification. Firstly, in a local manner, where acetylation is targeted to neighbouring nucleosomes of regulatory elements, in particular at enhancers and promoters but also at the most 5′-transcribed sequences. Secondly, in a less pronounced manner during the establishment of vast chromatin regions, which display an opened chromatin conformation (Calestagne-Morelli and Ausio 2006). The fact that such cell-type and context dependent domains of histone acetylation are

strictly conserved even between orthologous loci further suggest a strong selective pressure for proper inheritance of these PTM systems.

Importantly, it appears that the formation of silent chromatin regions is also dependent on the removal of active marks in such areas since the enzymes responsible for the formation of transcriptional repressive histone modifications, Suv39h1 and G9a for H3K9 methylation as well EED/EZH for K27 methylation and the silencing via CpG methylation are associated with HDAC activity (Jones *et al*. 1998; Nan *et al*. 1998; van der Vlag and Otte 1999; Fuks *et al*. 2000; Czermin *et al*. 2001; Vaute *et al*. 2002; Shi *et al*. 2003). Although HDAC inhibition is in most cases apparently not sufficient for reactivation of elements silenced by CpG methylation, inhibition of DNA methyltransferases was found to synergise with HDAC inhibitors (HDI) in the activation of such elements, suggesting a requirement for deacetylation in this silencing process (Kaslow and Migeon 1987; Cameron *et al*. 1999; Csankovszki *et al*. 2001). Further the maintenance of pericentric heterochromatin appears dependent on the suppressions of hyperacetylation, as HDAC inhibition results in delocalisation to the nuclear periphery and loss of HP1 retention (Taddei *et al*. 2005). Together these observations demonstrate an interplay between the placing of repressive marks and targeted suppression of activating marks in the formation and maintenance of transcriptional repressive chromatin states.

Interdependency between transcriptional activating lysine methylation and histone acetylation

Lysine methylation can also be associated with transcriptional active regions, as found for lysines 4, 36, 79 of histone H3 (Strahl *et al*. 1999; Santos-Rosa *et al*. 2002). Also in the case of activating methyl-lysine marks, methylation appears to be directly linked to acetylation. Recent reports demonstrate a tight coupling between methylation of H3K4 and lysine acetylation: Mass spectrometric studies indicated a preferential localisation of K4 methylation (mono-, di- and trimethylation) at penta-acetylated histone H3, whereas H3K9 methylation coincides mainly with mono-acetylation (Zhang *et al*. 2004). Mahadevan and colleagues reported a continuous turnover of histone acetylation at all K4 trimethylated histone H3 molecules, even during transcriptional silent states, which therefore renders these molecules hypersensitive to HDI mediated hyperacetylation (Hazzalin and Mahadevan 2005; Clayton *et al*. 2006). Further, transcriptional induction of the immediate early (IE) genes *c-fos* and *c-jun* required this continuous turnover rather than stable histone acetylation, which is in general associated with transcriptional active states, and interruption of the HAT/HDAC equilibrium ceased stimulus-dependent induction. HDAC inhibition and the resulting increase in acetylation did not affect the H3K4me3 levels at these genes suggesting that promoter associated nucleosomes are pre-modified to target acetylation turnover.

Nightingale *et al*. recently reported a global increase in H3K4 trimethylation upon HDAC inhibition and identified MLL4 as the responsible histone methyltransferases (Nightingale *et al*. 2006). Importantly, this stabilising effect of histone acetylation on H3K4 methylation was linked to an increased substrate-attraction of the H3K4 HMTase MLL1/4 by acetylated and phosphorylated histone H3 *in vitro*, whereas the activity of Set7, a HMTase mainly associated with H3K4-monomethylation was not affected (Milne *et al*. 2002; Xiao *et al*. 2003; Nightingale *et al*. 2006). The preference of MLL1/4 for acetylated substrates was demonstrated *in vitro* and in the absence of additional factors suggesting a direct impact of PTMs on the enzymatic activity (Nightingale *et al*. 2006). These findings are particular interesting as they demonstrate a reciprocal crosstalk between H3K4 methylation and lysine acetylation although in the case of *c-fos* and *c-jun* H3K4 methylation appears clearly independent of acetylation (Hazzalin *et al*. 2005). Strikingly, the HDI mediated increase in H3K4 trimethylation appears to be reversible upon loss of acetylation as removal of the HDI resulted in rapid deacetylation accompanied by a slower decline of H3K4me3 with a half life of roughly one hour suggesting the existence of a yet to be identified histone demethylase for this isoform. So far, only a demethylase with specificity for mono- and dimethylated H3K4 namely LSD1 was identified. Interestingly, LSD1 contains a nuclear localisation signal (NLS) and a SWIRM domain and is found, together with HDACs, in the Co-REST and other corepressor complexes (Lunyak *et al*. 2002; Lee *et al*. 2005) (**Fig. 1**).

Methylation of H3K4 was demonstrated to recruit several enzymatic activities (Sims and Reinberg 2006) including HAT and chromatin remodelling activity via Chd1, the BPTF subunit of the NURF complex and WDR5, which is also required for MLL activity (Santos-Rosa *et al*. 2003; Dou *et al*. 2005; Pray-Grant *et al*. 2005; Wysocka *et al*. 2005, 2006). Also the recently discovered H3K9/K36me3 histone demethylase JMJD2A is specifically recruited by H3K4me2/3 and H3K20me2/3 (Huang *et al*. 2006) and the PHD finger containing ING2 protein was identified as spe-

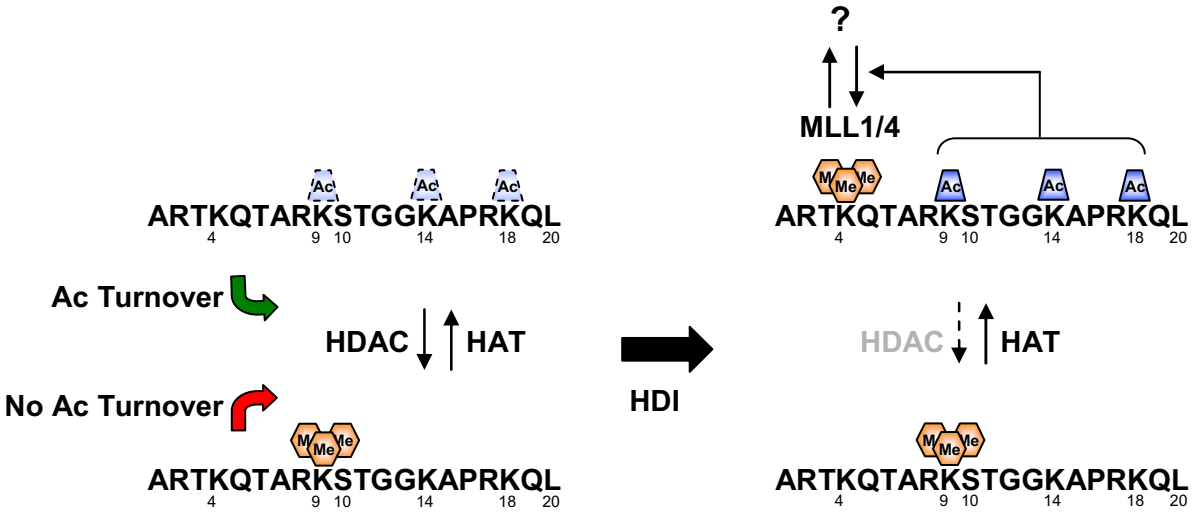

Fig. 1 Dynamic acetylation turnover at K4 methylated histone H3. The continuous reciprocal activities of HATs and HDACs are targeted to K4 methylated but not to K9 methylated histone H3. The former species displays therefore a hypersensitivity to HDAC inhibitor (HDI) mediated hyperacetylation, whereas K9 methylated histone H3 is less susceptible due to the missing activity of HATs. Histone acetylation in turn can exert a stabilising effect on K4 methylation by increasing the efficiency of MLL1/4 mediated K4 methylation, which can be reversed via a yet unidentified trimethyl-K4 specific demethylase.

cific detector-protein for H3K4me2/3 (Pena *et al*. 2006; Shi *et al*. 2006).

Besides the recruitment of transcriptional stimulating factors, H3K4 methylation can abolish the association or activity of transcriptional repressors such as the nucleosome remodelling and deacetylase complex (NuRD) or the H3K9 methyltransferase Suv39h1. Further, H3K4 methylation is essential for the restriction of Sir1p to heterochromatic regions, providing an additional level how this modification contributes to transcriptionally active chromatin (Nishioka *et al*. 2002; Zegerman *et al*. 2002; Santos-Rosa *et al*. 2004). Importantly, the ING proteins are associated with HDAC and HAT activity and can therefore recruit both transcriptional repressive and activating activities to H3K4me2/3 modified histone H3 (Doyon *et al*. 2006). Another example for the interaction with enzymatic activities that are normally associated with differential transcriptional effects is the co-localisation of HCF-1 with a Set1/Ash2 related HMTase complex as well as the Sin3 HDAC complex (Wysocka *et al*. 2003).

Histone phosphoacetylation, a special relation *in cis*

Histone phosphorylation comprises a particular interesting modification with extremely opposite characteristics in mitosis or interphase, respectively. Mitotic phosphorylation of histone H3 at serine 10 (H3S10ph) occurs in a global manner, first appearing in late G2 phase at pericentromeric heterochromatin and propagating throughout the whole chromosome until late prophase. Hyperphosphorylation is maintained during metaphase and dephosphorylation is initiated concomitant with anaphase and completed at telophase (Hendzel *et al*. 1997). This mitosis specific phosphorylation of histone H3 is mediated by the Aurora B kinase and is required for the displacement of HP1 proteins from mitotic condensed chromatin (Mateescu *et al*. 2004; Fischle *et al*. 2005; Hirota *et al*. 2005).

In contrast, interphase phosphorylation of histone H3 is a much more restricted event since only a few genomic regions are targeted by this modification. Importantly, this limited interphase histone phosphorylation correlates with transcriptional activation rather than chromatin compaction suggesting that the phosphorylation event itself does not directly initiate major alterations in chromatin structure.

Histone H3 phosphorylation was first discovered in the Sixties to be mediated by both cAMP-dependent and independent kinase activity and Mahadevan and co-workers described the phosphorylation of histone H3 as one of the earliest events in quiescent cells concomitant with the induction of the proto-oncogenes *c-fos* and *c-jun* upon growth factor or stress stimulation (Gutierrez and Hnilica 1967; Langan 1968; Shoemaker and Chalkley 1978; Mahadevan *et al*. 1991). This rapid and transient phosphorylation event on histone H3 is now referred to as the "nucleosomal response".

Since these reports histone H3 phosphorylation was found as a downstream target of several signal transduction cascades including the transcriptional activation of IE genes *c-fos* and *c-jun* as well as the late-induced *HDAC1* gene via MAP-kinase stimulation or the cytokine induced H3 phosphorylation via IKK-α (Thomson *et al*. 1999; Cheung *et al*. 2000; Clayton *et al*. 2000; Hauser *et al*. 2002; Anest *et al*. 2003; Yamamoto *et al*. 2003). Knockout studies indicate that H3S10 phosphorylation is mainly mediated via the activity of the downstream effector kinases MSK1/2. Besides other reports on the induction of histone H3 phosphorylation, which can not all be mentioned for reasons of brevity, it was demonstrated recently that also the progesterone receptor (PR) mediated transcriptional induction of the mouse mammary tumour virus (MMTV) promoter involves the phosphorylation of H3 in an ERK and Msk1 dependent manner (Vicent *et al*. 2006). In this report the authors demonstrated that the PR forms a ternary complex with ERK and MSK1 upon hormone stimulation, which in turn is rapidly recruited to the MMTV promoter to mediate H3S10 phosphorylation. Disturbance of this cascade and loss of histone phosphorylation resulted in impaired recruitment of the transcriptional co-activators Brg1 and PCAF and reduced localisation of RNA polymerase II (RNAP II) suggesting a role for the H3S10ph mark during the initiation phase of transcription.

Recently, another study investigated the osmotic-stress dependent repression of hormone-dependent transcription of the MMTV promoter. In this system, hormonal induction of MMTV promoter mediated transcription was rep-

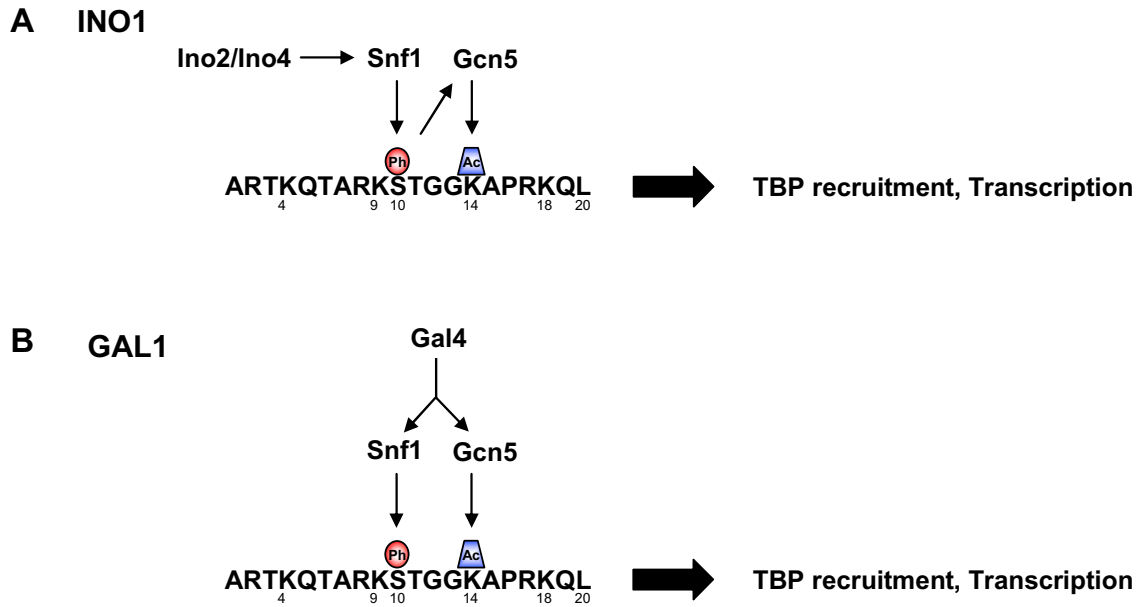

Fig. 2 Promoter specific crosstalk between histone H3 S10 phosphorylation and K14 acetylation. Transcriptional induction of the *INO1* and the *GAL1* genes involves phosphoacetylation of histone H3. (A) The Ino2/4 heterodimer interacts with the Snf1 kinase and recruits the enzyme to the *INO1* TATA sequence where it phosphorylates histone H3 at S10. The phosphorylation event is required for the subsequent recruitment of the Gcn5 HAT and acetylation of K14. (B) In contrast to the *INO1* activator Ino2/4, the *GAL1* activator Gal4 can interact with both Snf1 and Gcn5 and therefore acetylation of K14 at the *GAL1* promoter is independent of S10 phosphorylation. Despite this uncoupling, both histone modifications are required for TBP recruitment and transcriptional induction at both promoters.

ressed by osmotic shock in UL3 cells. Osmotic-shock mediated displacement of the glucocorticoid receptor (GR) temporarily and spatially coincided with phosphorylation of H3S10 and H3S28 at histone H3 suggesting that interphase phosphorylation of H3 can also occur during transcriptional repressed states in this system (Burkhart *et al.* 2007).

An interesting feature of H3S10 phosphorylation during interphase is a strong link with acetylation events on neighbouring lysines at position 9 and 14 (H3K9/14ac), which finally gave rise to the neologism "histone H3 phosphoacetylation" (H3S10ph/K14ac) (Cheung *et al.* 2000; Clayton *et al.* 2000). The explanation for the close co-existence of both PTMs is controversial. The observation that the transcriptional co-activator and HAT Gcn5 displayed a strongly increased preference for histone H3 peptides when they were phosphorylated at S10, and Snf1 kinase mediated H3S10 phosphorylation was required for the additional acetylation of H3K14 at the INO1 promoter led to the proposal of a "synergistic coupled model" (Cheung *et al.* 2000; Lo *et al.* 2000, 2001). According to this scenario, the preceding phosphorylation increases the affinity of HAT-enzymes for the histone H3 tail and is obligate for the subsequent acetylation event to generate the phosphoacetylated isoform. Indeed studies on the substrate preferences of recombinant Gcn5 with uniformly H3S10 phosphorylated nucleosomal arrays confirmed these results. However, this preference for H3S10ph nucleosomes was abolished when Gcn5 was assembled into the native SAGA complex (Shogren-Knaak *et al.* 2003). Studies on two Snf1 regulated targets genes, INO1 and GAL1 revealed a gene specific validity of this model. Whereas H3S10ph at the INO1 promoter was required for recruitment of the SAGA complex and subsequent acetylation of H3K14, at the GAL1 promoter both events are independent and SAGA is recruited also in the absence of phS10 (Lo *et al.* 2005). Importantly, the relevant activators showed different abilities to interact with the co-activators Snf1 and SAGA, respectively; INO1

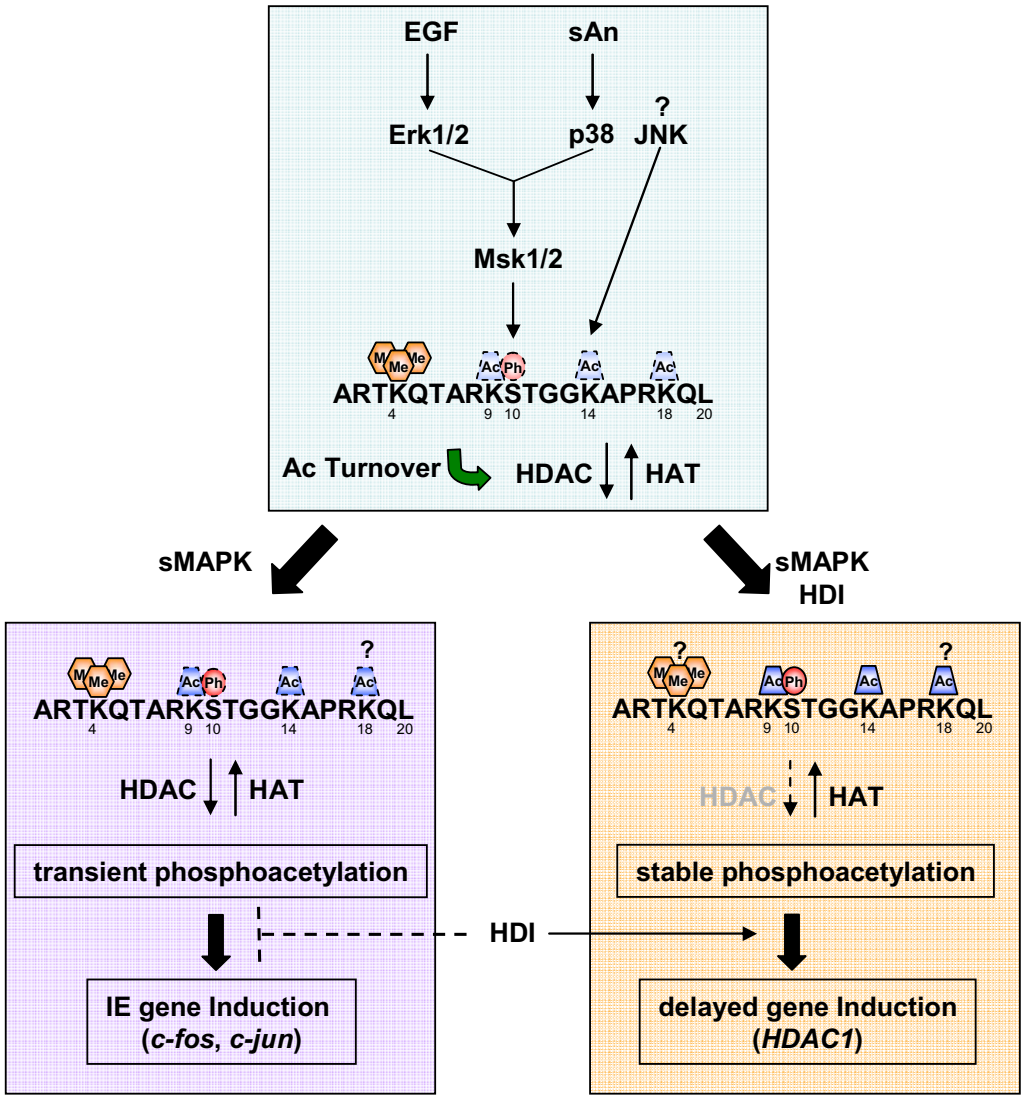

Fig. 3 Phosphoacetylation of histone H3 impacts transcription by gene specific mechanisms. (Upper panel) Two MAP kinase cascades, the ERK and p38 pathways are upstream of S10 phosphorylation. ERK can be activated by growth factors like EGF and p38 by stress inducers like anisomycin. Both cascades can mediate activation of the effector kinase MSK1/2 that directly phosphorylates histone H3 (red circle). An alternative effector kinase may be Rsk2, which is only activated via the ERK pathway. S10 phosphorylation is targeted to a minute fraction of nucleosomes that are highly susceptible to lysine acetylation and in the case of *c-fos* and *c-jun* both modifications are targeted to K4 methylated nucleosomes, which are subjected to continuous acetylation turnover (blue trapezium) (see also **Fig. 1**). In contrast to ERK and p38, the JNK pathway does not mediate S10 phosphorylation. However, it was suggested that JNK could mediate the increase in lysine acetylation at the *c-fos* and *c-jun* promoters observed upon JNK/p38 stimulation independently of S10 phosphorylation. (Left panel) Transcriptional induction of IE genes, which are "poised to transcribe", is mediated via transient phosphoacetylation mediated by MAP-kinase stimulation. S10 phosphorylation and acetylation are independently localised at K4 methylated nucleosomes and blockade of acetylation turnover via HDI ceases transcriptional induction. (Right panel) Transcriptional induction of the non-IE phosphoacetylation target gene *HDAC1* requires both modifications in a stable manner. Histone phosphoacetylation can be stabilised via additional HDAC inhibition (HDI) and both modifications show synergy in the transcriptional induction of *HDAC1* (for details see text). The role of K4 methylation has not yet been determined in this system.

activator can solely recruit Snf1 kinase activity, whereas GAL4 activator can interact with both factors explaining the uncoupling of H3S10ph and H3K14ac at the GAL1 promoter. However, despite this lack of direct dependency, H3S10ph was still required to mediate transcriptional induction of the GAL1 and the INO1 gene suggesting a cooperation of H3S10ph and H3K14ac in transcriptional activation (**Fig. 2**).

Additional support for an independent recruitment of both modifications comes from detailed studies on the IE *c-fos* and *c-jun*. Upon MAP-kinase stimulation via growth factors or stress inducers like anisomycin, only a minute fraction of nucleosomes becomes phosphorylated in quiescent cells which is particular susceptible to HDI induced hyperacetylation (Barratt *et al*. 1994). The nucleosomes of the *c-jun* promoter region are rapidly and transiently phosphoacetylated and appearance of this dual-modified isoform is strongly correlated with transcriptional induction. Thomson and co-workers found that the regulation of H3S10ph and H3K9/14ac is dynamically and independently regulated at the promoters of these protooncogenes (Thomson *et al*. 2001). Both modification events occur independently as inhibition of p38 or ERK MAP-kinase activity abolished H3S10ph and H3S10ph/K14ac but did not alleviate anisomycin induced hyperacetylation (Thomson *et al*. 2001; Mahadevan *et al*. 2004) (**Fig. 3**). This suggests further that another stress-responsive pathway mediates the local equilibrium-shift between HAT and HDAC activity. Importantly, p38 MAP-kinase stimulation did not influence bulk histone acetylation levels, implying the strict local delivery of this effect (Hauser *et al*. 2002). In addition HDI induced histone H3 hyperacetylation was observed in G0 cells at the bulk chromatin level and the *c-jun* promoter, demonstrating a continuous turnover of this modification even in the absence of H3S10ph (Thomson *et al*. 2001; Hauser *et al*. 2002). Despite a stable bulk histone hyperacetylation in the presence of TSA, histone acetylation at the IE genes is transient, and may therefore involve a TSA-insensitive deacetylase (Thomson *et al*. 2001). A striking feature of this continuous acetylation turnover at the *c-fos* and *c-jun* promoters was described recently: ablation of this turnover via HDI ceases MAP-kinase mediated transcriptional induction of these genes, demonstrating that constant acetylation/deacetylation is required for activation rather than a highly acetylated state (Hazzalin *et al*. 2005). This requirement of acetylation turnover appears to be a specific trait of the IE genes as deacetylase inhibition mainly exerts a positive transcriptional response, although some genes clearly require HDAC activity for expression (Zupkovitz *et al*. 2006).

Although in the case of IE genes, phosphorylation and acetylation of histone H3 constitute two independent events, both modifications were found to synergise in the transcriptional activation of the late inducible HDAC1 gene, which is therefore differentially regulated as the IE genes (Hauser *et al*. 2002). HDAC1 is repressed via a negative feedback mechanism and not expressed in serum starved G0 cells (Hauser *et al*. 2002; Schuettengruber *et al*. 2003). The local HDAC predominance can be overcome, by HDI treatment and was sufficient to induce low levels of HDAC1 expression. Although MAP-kinase activation leads to transient phosphoacetylation at the HDAC1 promoter, this short-term alteration in chromatin structure is not sufficient to overcome the transcriptional repressive, local predominance of HDACs. Upon additional inhibition of deacetylase activity phosphoacetylation is stabilised and shows synergistic effects on the transcriptional induction (Hauser *et al*. 2002) (**Fig. 3**). This study did not address the question concerning coupled or uncoupled phosphoacetylation, but clearly demonstrates that histone phosphorylation and acetylation can have synergistic effects in transcriptional activation.

Another interesting feature of H3S10 phosphorylation is the strong inhibition of the H3K4 lysine specific demethylase LSD1. The observations that LSD1 mediated H3K4 demethylation is strongly dependent on removal of other PTMs suggests that several enzymatic activities cooperate during the formation of transcriptional repressive states (Forneris *et al*. 2005, 2006).

The function of histone phosphorylation in transcriptional induction is still not understood. However, due to the extreme local restriction of this modification, and the variety of signal transduction systems that have been reported to mediate histone phosphorylation it is likely that this PTM can mediate transcriptional induction via several different mechanisms. Therefore, the genomic context of H3S10 phosphorylation may be a critical factor for the molecular events, initiated by this modification.

Histone ubiquitination and modification crosstalk *in trans*

Ubiquitination of histone molecules was found for histones H2A, H2B, H3, H4, H2A.Z, macroH2A and H1 (Zhang 2003; Kinyamu *et al*. 2005; Osley *et al*. 2006). In most cases only a single ubiquitin molecule is attached to histones, which is not sufficient for targeting via the 26S proteasome.

Ubiquitination of Histone H1 is mediated via the $TAF_{II}250$ component of the TFIID complex and is linked to the transcription of a subset of genes. Ubiquitination via $TAF_{II}250$ is particularly interesting as this is the only known factor that possesses both, ubiquitin activating (E1) and conjugation (E2) activity (Pham and Sauer 2000).

Histone H2A, the first protein found to be ubiquitinated (Goldknopf *et al*. 1975), is modified at position K119 within the C-terminal globular histone fold domain. Ubiquitinated H2A (H2AK119ub1) comprises approximately 5-15% of total cellular H2A molecules but seems to be restricted to higher eukaryotes and is apparently absent in *Saccharomyces cerevisiae*. Histone H2A ubiquitination is mediated via RING-domain containing components of the polycomb group complex PRC1 and was functionally linked to polycomb silencing and X chromosome inactivation (de Napoles *et al*. 2004; Fang *et al*. 2004; Wang *et al*. 2004). On the other hand the same modification was found to correlate with transcriptionally active chromatin states, exemplified by an enrichment of H2AK119ub1 on the nucleosomes of the *hsp70* promoter and a depletion at non-transcribed satellite DNA (Levinger and Varshavsky 1982). Further, ubiquitination of H2A and H2B was shown to be enriched at actively transcribed regions in different systems like bovine thymus, chicken erythrocytes and macronuclei of *Tetrahymena* (reviewed in Zhang 2003 and Osley *et al*. 2006).

Ubiquitination of H2B was found at K123 in yeast and at K120 in other eukaryotes. In contrast to H2A, H2B ubiquitination is much less abundant (1-2% of total H2B molecules) but more widely distributed within eukaryotes. The yeast enzymes responsible for H2B ubiquitination have been identified as the E2 ligase Rad6/Ubc2 and the E3 ligase Bre1, and both these enzymes have human homologues, UbcH6 and RNF20/RNF40, respectively (Zhang 2003; Osley *et al*. 2006) (**Fig. 4**). Rad6/Ubc2 represents a multifunctional E2 enzyme with several different cellular substrates that are specifically targeted via the interaction with different E3 ligases. Bre1 is an important co-factor for Rad6 mediated ubiquitination, as it is also required for the recruitment of Rad6 to promoters, as well as its association with the elongating RNAP II and spreading into the coding region (Henry *et al*. 2003; Wood *et al*. 2003). A multitude of additional factors that promote H2B ubiquiti-nation have been described and a majority of them are in-volved in transcriptional regulation as will be discussed below.

It is noteworthy that both histone H2A and H2B constitute (although inefficient) targets for Mdm2 E3 ligase mediated mono-ubiquitination and therefore alternative E3 ligases may provide a gene-specific context for H2A/H2B ubiquitination (Minsky and Oren 2004).

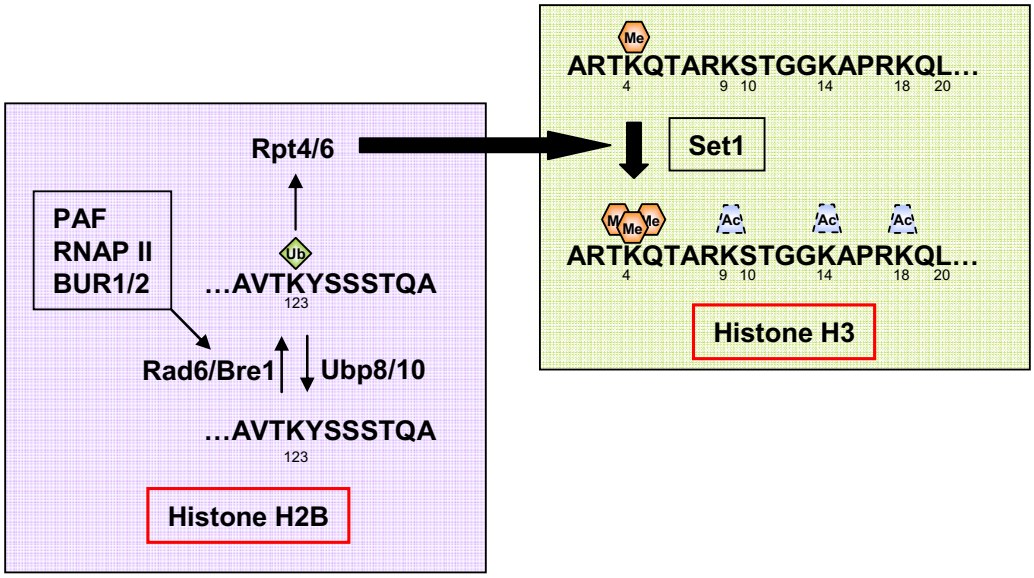

Fig. 4 Modification crosstalk *in trans* between H2B ubiquitination and H3 methylation. Ubiquitination of H2B at K123 in yeast (K120 in human) was found as essential upstream event for methylation of K4 at histone H3. Rad6/Bre1 were identified as responsible E2 and E3 ligases, respectively. The isopeptidase Ubp8 removes ubiquitin in euchromatic regions, whereas Ubp10 mainly acts on heterochromatic chromatin. Rad6/Bre1 mediated ubiquitination was found to be dependent on various additional factors including the RNAP II/PAF elongation complex, as well as the activity of BUR1/2 kinase. Monoubiquitination of H2B is not required for recruitment of the K4 HMTase Set1, but for the localization of the proteasomal components Rpt4/6, which in turn are required for processive di- and trimethylation of K4. K4 methylation again may direct acetylation turnover to the H3 tail *in cis* (see also **Fig. 1**).

The responsible enzyme for removing ubiquitin from H2B in euchromatic regions was identified as Ubp8 a component of the SAGA acetyltransferase complex, which therefore combines deubiquitination and acetylation activities (**Fig. 4**) (Henry *et al*. 2003; Daniel *et al*. 2004). Importantly, efficient transcriptional initiation requires sequential ubiquitination and deubiquitination as presence of uH2B coincides with the localisation of RNAP II and absence of this modification results in a delay of this recruitment. Further, it was found that the loss of Ubp8 results in an inverse imbalance of H3K4 and H3K36 methylation patterns, which may also perturb transcription (Henry *et al*. 2003).

Another ubiquitin protease that targets H2B is Ubp10, which appears to primarily act on subtelomeric regions adjacent to heterochromatin, although a role in euchromatic repression has bee observed (Emre *et al*. 2005; Gardner *et al*. 2005) (**Fig. 4**).

Genetic analysis of yeast mutants displaying defects in H2B ubiquitination revealed an amazing dependency of H3K4 and H3K79 methylation on this modification. Mutations that abolish ubiquitination of H2B also globally affected H3K4 and H3K79 methylation and caused telomeric and mating type locus silencing defects. This loss of heterochromatic structures was linked to the decreased repulsion of Sir proteins from euchromatic regions due to impaired H3K4 methylation (Briggs *et al*. 2002; Dover *et al*. 2002; Sun and Allis 2002). Recent data indicate that monomethylation of H3K4 is independent of H2B ubiquitination whereas the di- and tri-methylated states are preferentially regulated *in trans* (Dehe *et al*. 2005; Shahbazian *et al*. 2005). One key component in this modification crosstalk seems to be the transcription elongation regulator complex PAF and the association of HMTases and ubiquitin ligases with elongating RNAP II (Krogan *et al*. 2003; Ng *et al*. 2003).

Importantly, ubiquitination of H2B is not determinant for the recruitment of Set1 and Dot1 HMTases, but required for the localisation of the proteasomal ATPases Rpt4 and Rpt6, which are in turn essential for histone H3 methylation (Ezhkova and Tansey 2004) (**Fig. 4**). Further the Rpt6 ATPase activity of the 19S proteasome regulatory particle (19S RP) was found to be necessary for optimal recruitment of the SAGA-complex to the GAL1-GAL10 promoters, which demonstrates a non-proteolytic role for proteasomal components in transcriptional activation (Lee *et al*. 2005). SAGA associates with the chromodomain protein Chd1 that binds methylated K4, and the complex integrates histone acetyltransferase and ubiquitin protease activities via the components Gcn5 and Ubp8. Both histone ubiquitination and acetylation are dynamically regulated during transcriptional initiation by SAGA demonstrating the importance of the 19S RP mediated regulation of SAGA recruitment.

Besides the PAF elongation complex the transcription elongation factor BUR1, a cyclin-dependent type kinase (cdk), in conjunction with the cyclin BUR2, was found as an essential component for histone H2B ubiquitination, H3K4 trimethylation and mediator for the interaction with the PAF-complex (Laribee *et al*. 2005; Wood *et al*. 2005). Interestingly, mutation of H3K36 to arginine or defects in the H3K36 specific HMTase Set2, were identified as origins of the growth defect displayed by BUR1/BUR2 mutants. These mutants also displayed reduced levels of trimethylated H3K36 suggesting that the regulatory kinase BUR1/BUR2 also influences transcription-dependent H3K36me3 via Set2 (Chu *et al*. 2006).

How exactly ubiquitination impacts transcription is still elusive since some defects displayed in uH2B mutants may be contributed by the crosstalk with H3K4/79 methylation. However, there is accumulating evidence for a function of uH2B in transcriptional initiation and elongation some of which may also be mediated via downstream methylation and some directly contributed via the ubiquitin mark. One model for the role of histone ubiquitination proposes that the effects of this modification are mainly exerted via the bulky ubiquitin moiety and the impact of this residue on the overall chromatin structure. However, *in vitro* studies failed to detect any major alteration in the structure of nucleosomal arrays. The second model proposes that ubiquitin serves as binding site for modification-dependent detector proteins, which recognise the ubiquitin moiety and lead to the recruitment of further transcriptional activators.

CHROMATIN REMODELLING COMPLEXES

Chromatin and especially nucleosomes are thought to be highly dynamic in response to several biological processes. It is becoming increasingly clear that nucleosomes do not just provide for packing the DNA within chromatin, but are exploited to regulate biological processes in very sophisticated ways. The simplest way by which repression can be achieved is the formation of a stable and inaccessible chromatin structure (heterochromatin). In contrast, activation can be attained by creating an accessible chromatin structure (euchromatin). Upon transcriptional activation nucleosomes often have to be repositioned or even ejected to allow transcription factor binding and recruitment of the transcription machinery (Bernstein *et al*. 2004). ChIP-on-chip experiments using antibodies specific for core histones provide further insights into the location of nucleosomes. Genome-wide studies in yeast suggest an inverse relation between nucleosome density at promoters and transcriptional activation (Bernstein *et al*. 2004; Lee *et al*. 2004). Additionally, it was shown that highly transcribed genes are partially depleted of nucleosomes within the coding region (Lee *et al*. 2004).

Due to the numerous histone-DNA contacts (14 hydrogen bonds and salt linkers) nucleosomes are very stable protein-DNA complexes. Therefore, their displacement normally requires a net-energy input, which is supplied by ATP hydrolysis. ATP-dependent chromatin remodelling complexes in combination with histone chaperones and DNA-binding transcription factors facilitate the displacement of nucleosomes by "sliding" of the DNA with respect to the histone octamer (Meersseman *et al*. 1992; Becker 2002; Kassabov *et al*. 2003; Zofall *et al*. 2006). Additionally, chromatin remodelling may even lead to the transition of the nucleosome to a different DNA segment (nucleosome transfer) (Lorch *et al*. 1999; Korber *et al*. 2004). The traversing RNA polymerase itself was also shown to possess ATP-independent remodelling activity (Studitsky *et al*. 2004). However, it is tempting to speculate that the elongating RNA polymerase acts in concert with ATP-dependent chromatin remodelling complexes, histone chaperones and co-activator complexes upon activation of gene transcription.

The challenges of exposing DNA differ according to local nucleosome organisation. Therefore, it appears that nature has evolved different classes of ATP-dependent remodellers that use distinct mechanisms, each suited to a specific biological task. All known ATP-dependent chromatin remodelling enzymes belong to the helicase superfamily 2 (SF2). Up to now 5 classes of multi-subunit ATP-dependent remodelling complexes are distinguished, based on the identity of their catalytic ATPase subunit, different protein-complex compositions and functional diversity (**Table 1**). For excellent reviews on ATP-dependent remodelling complexes in higher eukaryotes see (Corona and Tamkun 2004; Langst and Becker 2004; Bouazoune and Brehm 2006; Saha *et al*. 2006).

(1) One of the best studied is the SWI/SNF (switch 2/ sucrose-non-fermenting 2) family, which includes the yeast SWI2/SNF2 (ySWI/SNF), the yeast complex "remodels the structure of chromatin" (RSC), the *Drosophila* Brahma complexes BAP and PBAP, the human BRM (hBRM) and BRG1 (hBRG1), and the mouse BRG1 (mBRG1) complexes (Aoyagi *et al*. 2002).

(2) The ISWI (imitation switch) family complexes are smaller and have fewer subunits than their SWI/SNF counterparts. The most extensively studied members of this group in *Drosophila* are the "ATP-utilizing chromatin assembly and remodelling factor" (ACF), "nucleosome-remodelling factor" (NURF), and "chromatin accessibility complex" (CHRAC) (Corona *et al*. 2004; Mellor and Morillon 2004).

(3) The Mi-2 or CHD family contains remodelling and HDAC activities. In human the best studied is the "nucleosome remodelling and deacetylase" (hNURD) complex, which contains HDAC1 and -2, the retinoblastoma protein (Rb)-associated proteins RbAp46 and RbAp48, and the Swi2/Snf2 ATPase homologue CHD4, also known as Mi-2 (antigen recognised by the patient Mitchell autoimmune antibodies 2) (Tong *et al*. 1998)).

Table 1 ATP-dependent chromatin remodeling complexes of the helices superfamily 2 (SF2).

Family	ATPase	Characeristics	Organism	Complexes	Function	References
SWI/SNF	BRM/ BRG1	Bromodomain	Hs Mm	hSWI/SNF mSWI/SNF	Transcriptional activation	Trotter and Archer 2007
	BRM	Bromodomain	Dm	BAP PBAP	Transcriptional activation (elongation?)	Mohrmann 2004 Bouazoune and Brehm 2006
	Swi1/Snf2	Bromodomain	Sc	SWI/SNF	Transcriptional activation/repression	Martens 2002; Chandy 2006
	Sth1			RSC	Transcriptional elongation	Carey 2006
ISWI	ISWI	SANT domain SLIDE domain (PHD-finger (ACF1))	Hs	WCRF/hACF WICH hCHRAC RSF	Transcriptional activation/repression	Corona and Tamkun 2004
			Mm	NoRC mWICH	Transcriptional activation/repression	
			Dm	ACF CHRAC NURF	Higher chromatin structure Transcriptional activation/repression Replication	Bouazoune and Brehm 2006 Corona and Tamkun 2004
			Sc	ISW1a/b ISW2	Transcriptional elongation/termination Transcriptional repression	Mellor and Morillon 2004
Mi-2	Mi-2β/CHD4	Chromodomain PHD-finger	Hs (Mm)	NuRD	Transcriptional repression	Bowen *et al*. 2004
	dMi-2	Chromodomain PHD-finger	Dm	dNuRD	Transcriptional repression Development	Bouazoune and Brehm 2006
SWR1	Swr1p	Split ATPase domain	Hs	SRCAP	H2A.Z chaperone	Cai 2005
			Dm	Domino	H2A.Z chaperone	Eissenberg 2005
			Sc	SWR1	H2A.Z chaperone	Mizuguchi 2004
INO80	Ino80p	Split ATPase domain	Hs	hINO80	DNA repair	Jin 2005
			Dm	dINO80	DNA repair	
			Sc	INO80	DNA repair	Morrison and Shen 2005

(4 and 5) In addition to these three families, two other chromatin remodelling complexes, referred to as SWR1 and INO80 have been known in yeast, and only recently complexes containing proteins with striking similarity to SWR1 and INO80 respectively, have been discovered in human. The mammalian "SWI/SNF-related CBP activator protein" (SRCAP) resembles the yeast SWR1 chromatin remodelling complex and contains proteins, including TIP49a and TIP49b, which are also subunits of the TRRAP/TIP60 HAT complex described by Conaway and colleagues (Cai *et al*. 2005). The same group purified a mammalian INO80-related remodelling complex from HeLa cells, referred to as hINO80, which consists of several orthologues of subunits of the yeast INO80 complex, including once more TIP49a and TIP49b (Jin *et al*. 2005).

Recruitment of chromatin remodelling activity

Remodelling complexes mediate and regulate a remarkable number of biological processes and therefore need to be highly specialised. For example ISWI complexes were shown to have central roles in repressive chromatin assembly, which involves regular and constant nucleosome ordering and spacing. In contrast, SWI/SNF complexes are responsible to disorder and reorganise nucleosomes to increase DNA accessibility upon gene activation (reviewed in (Corona *et al*. 2004; Dirscherl and Krebs 2004; Saha *et al*. 2006). However, SWI/SNF complexes were also shown to be involved in transcriptional repression (Martens and Winston 2002), while ISWI complexes are also involved in transcriptional activation (Deuring *et al*. 2000). It is believed that remodelling complexes achieve their specificity mainly due to the binding to sequence specific transcription factors in a particular chromatin context. Binding can be even stimulated and increased by motifs that enable the recognition of special chromatin (see **Table 1**). For example the ISWI complex interacts with the Acf1 protein, which can bind to the histone core via a PHD domain (Ragvin *et al*. 2004). In addition, it contains two domains, known as the SANT and SLIDE domain, on the C-terminus, which recognise especially the histone H4 tail and the linker DNA that emits from the nucleosome, respectively (Grune *et al*. 2003).

Other remodelling complexes, as the SWI/SNF and RSC complexes, bind to nucleosomes via one or more bromodomains with a preference for particular acetylated residues on the histone tail. For instance the RSC complex is highly specific for H3K14ac (Kasten *et al*. 2004). In contrast, Bdf1 a component of the SWR1 complex, was shown to preferentially bind histone H4 acetylated at H4K5, H4K8 and H4K12, but not H4K16 (Kurdistani *et al*. 2004). Members of the Mi-2 family might recognise repressive chromatin by a chromodomain (Kelley *et al*. 1999). Therefore, it is not astonishing that remodelling complexes operate along with histone modifying complexes, like histone acetyltransferase complexes (SAGA, NuA3 and NuA4) and histone deacetylase complexes (Sin3 and NuRD).

Models for chromatin remodelling

Due to the many different biological tasks, it is tempting to assume that remodelling complexes might use distinct and probably unique mechanisms of remodelling. However, extensive *in vitro* and *in vivo* studies indicate that all different phenomena of remodelling can be explained by variations of one and the same model. Several possible models for chromatin remodelling have been discussed and most of them are related to each other and differ only in minor variations (Studitsky *et al*. 2004; Saha *et al*. 2006).

The "bulging/looping model" involves the unwrapping of the DNA from the nucleosome edge to form a DNA bulge/loop, which is then moved around the nucleosomal surface. In this model, nucleosome/DNA contacts are broken on one side of the bulge and reformed on the other side. The propagation of the DNA bulge around the histone octamer can lead to nucleosome sliding, a displacement of the octamer *in cis*. As the formation of a DNA bulge leads to a partly accessible octamer surface, the detached DNA cannot only be replaced by neighbouring DNA, but also by an unconnected DNA molecule enabling an octamer transfer *in trans*.

The "wave model" is very similar to the "bulge model" and suggests that DNA is "pumped" around the nucleosome, resembling a DNA wave. The resulting loss of contact often leads to an exchange of a histone H2A-H2B dimer, which in turn allows for the incorporation of an H2A.Z-H2B dimer (Bruno *et al*. 2003; Zhang *et al*. 2005) or eviction of the whole nucleosome (Lee *et al*. 2004). Evicted nucleosomes are frequently exchanged by replacement variant H3.3 containing nucleosomes, a hallmark of active chromatin in higher eukaryotes (Wirbelauer *et al*. 2005). In this model nucleosomes are replaced *in cis* and *in trans*.

According to the "twisting model" torsion applied to DNA could lead to DNA twisting and disruption of histone-DNA contacts; this model actually involves both mechanisms described above (Studitsky *et al*. 2004).

Other authors have suggested slightly different models, which however, are variations of the described models. For instance the "loop recapture model" and the "wave-ratchet-wave model" are discussed for the SWI/SNF and ISWI complexes in recently published reviews (Langst *et al*. 2004; Saha *et al*. 2006).

The ySWI/SNF remodelling complex

The yeast SWI/SNF ATP-dependent chromatin remodelling complex was first identified and characterized over 10 years ago (Winston and Carlson 1992). Even though remodelling by the SWI/SNF complex is one of the best studied, the mechanism of its action is not completely understood. Due to the low number of SWI/SNF complexes within a cell compared to the vast number of nucleosomes that need to be remodelled and the dual function of this complex, which is involved in both transcriptional activation and repression, it was suggested that chromatin remodelling by SWI/SNF is a tightly regulated process and is restricted predominantly to promoter regions of active genes (Peterson and Workman 2000).

A large number of *in vitro* studies revealed that SWI/SNF catalyses nucleosome sliding (Kassabov *et al*. 2003; Zofall *et al*. 2006), whereas *in vivo* data increasingly suggest SWI/SNF to be involved in nucleosome eviction (also referred to as nucleosome transfer *in trans*) at activated promoters (Reinke and Horz 2003; Korber *et al*. 2004). It was shown that the yeast SWI/SNF remodelling complex preferentially removes histones that have been acetylated by the SAGA complex from nucleosomal arrays *in vitro* (Chandy *et al*. 2006). This observation can be linked to the finding that nucleosome displacement by SWI/SNF is at least partly dependent on the SWI/SNF bromodomain. In addition to PTMs the activity of SWI/SNF is enhanced by binding to transcription factors carrying an activation domain *in vivo* (Gutierrez *et al*. 2007). Due to these findings and the fact that many promoters of activated genes are nucleosome-free the following mechanism was suggested: SAGA complex is recruited to promoter nucleosomes, which are acetylated by the SAGA histone acetyltransferase Gcn5. Binding sites get accessible for transcription factors which finally target SWI/SNF complex to the SAGA acetylated nucleosomes. The displacement of these nucleosomes by SWI/SNF generates a docking site for the RNAP II transcription machinery (Chandy *et al*. 2006; Gutierrez *et al*. 2007). It is worth mentioning that, in addition to transcriptional activation, SWI/SNF complexes were shown to be involved also in transcriptional repression (Martens *et al*. 2002).

The hBRG1 chromatin remodelling complex

In mammals, the SWI/SNF homologue BRG1 was also found to participate in nuclear receptor (NR) mediated transcriptional regulation (Trotter and Archer 2007). The steroid hormone inducible MMTV promoter is a well-established model system and was used extensively to study the role of SWI/SNF in NR-mediated gene activation. GR and estrogen receptor (ER) interact with SWI/SNF subunits and are most likely responsible for site-specific recruitment of the remodelling complex. Subsequent remodelling of the MMTV promoter leads to increased accessibility of hormone response elements (HREs) and increased NR binding with concomitant gene activation. Moreover, ISWI and Mi-2 based remodelling complexes cannot activate GR-dependent target genes, suggesting that BRG1 is the responsible remodelling complex required for hormone-dependent transcription (Trotter *et al*. 2007). Further studies showed that GR binding and remodelling by BRG1 is of transient and cyclical nature, which involves reorganisation of especially H2A and H2B histones (Nagaich *et al*. 2004).

As mentioned above, BRG1 recruitment to the MMTV promoter is not only dependent on GR binding but also on phosphorylation of S10 on histone H3 (Vicent *et al*. 2006).

In contrast to gene activation, human SWI/SNF plays also a role in chromatin condensation and gene repression. For instance BRG1 and hBRM were found to participate in Rb-E2F repression pathways (Zhang and Dean 2001), and Sin3, a HDAC complex, was shown to interact *in vitro* with components of the hSWI/SNF complex (Sif *et al*. 2001).

The yRSC chromatin remodelling complex

Another example of coordinated action of HATs and ATP-dependent remodelling complexes involves RNAP II elongation. The RSC remodelling complex was shown to facilitate RNAP II elongation on nucleosomes, which was further enhanced by acetylation of nucleosomes by SAGA and NuA4 HAT complexes (Carey *et al*. 2006). RSC possesses in total seven bromodomains, which recognise acetylated form of K14 on histone H3, the main target residue of Gcn5 within the SAGA complex (Kasten *et al*. 2004). Moreover, RSC can directly interact with RNAP II via the RPB5 subunit (Soutourina *et al*. 2006).

However, the function of histone acetylation during transcriptional elongation is more complicated, especially due to the fact that coding regions are actively deacetylated by Rpd3 (Lieb and Clarke 2005). Deacetylation is a consequence of K36 methylation and is required to prevent transcriptional initiation from cryptic promoters within the coding region (Carrozza *et al*. 2005). It is believed that acetylation and RSC recruitment is restricted to nucleosomes preceding the elongating RNAP II machinery. This would permit the required remodelling of chromatin ahead of RNAP II. Deacetylation of histones after RNAP II passage would prevent chromatin remodelling and nucleosome eviction (Carey *et al*. 2006). Therefore, it seems that a tight regulation of acetylation and deacetylation during elongation is of ultimate importance to guarantee the maintenance of chromatin stability and integrity.

ISWI chromatin remodelling complexes

ISWI was discovered in a screen for brahma related genes in *Drosophila* (Elfring *et al*. 1994) and was subsequently found as component of three chromatin remodelling complexes. ISWI homologues were identified in many other organisms including yeast and mammals, suggesting that this ATPase is highly conserved. Functions of ISWI chromatin remodelling complexes are diverse and range from transcriptional regulation to global chromosome organisation and DNA replication (Corona *et al*. 2004).

Even though there is evidence for a role in transcriptional activation, ISWI containing complexes are definitely involved in repression. For instance, in *Drosophila* polytene chromosomes ISWI complexes do not overlap with RNAP II, but are predominantly found in silent areas (Deuring *et al*. 2000). Additionally, there is direct evidence that yeast ISWI complexes, in particular Isw1a and Isw2, are involved in transcriptional repression (Goldmark *et al*. 2000; Morillon *et al*. 2003). In contrast, the Isw1b complex was found to play a role in transcriptional elongation and termination (Morillon *et al*. 2003).

ISWI complexes are also involved in chromosome organisation. For instance, in the absence of ISWI the male X chromosome in *Drosophila* larvae appears much less condensed. In *Drosophila* dosage compensation is accomplished by increasing the level of transcription of the single dose of X-linked genes in males. This is partly achieved by hyperacetylation of H4K16 by the histone acetyltransferase MOF, a component of the dosage compensa-tion machinery (Park and Kuroda 2001). When H4K16 acetylation is blocked, the phenotype of the male X chromosome in *ISWI* mutants is rescued, suggesting that histone H4K16ac directly counteracts chromatin compaction mediated by the ISWI ATPase (Corona *et al*. 2004).

The NuRD chromatin remodelling complex

The NuRD complex was found to associate with several sequence specific transcriptional repressors, which might be responsible for local targeting of the complex. In addition, the complex might be recruited via the interaction with methylated lysines through chromodomain containing subunits. However, no binding to methylated H3K9 or H3K4 was detected, suggesting that NuRD might recognise a different methylated lysine residue (Flanagan *et al*. 2005).

Another indication for a role in transcriptional repression is the ability of *Drosophila* NuRD to bind to DNA methylated at CpT/A sequences via its methyl binding domain (MBD)-subunits. Finally, chromatin remodelling by NuRD facilitates deacetylation, (one further hallmark of silenced and transcriptional repressed chromatin) due to the HDAC activity within the complex (Tong *et al*. 1998). However, other studies suggested a connection of dMi-2 complexes with active gene transcription, as dNuRD colocalises with active forms of RNAP II on polytene chromosomes (Srinivasan *et al*. 2005). This discrepancy can be explained by recent studies which suggested a very dynamic acetylation and deacetylation behaviour together with extensive chromatin remodelling during transcriptional elongation (Ekwall 2005).

The ySWR1 chromatin remodelling complex

Several chromatin remodelling complexes including SWI/SNF, RSC and ISWI, can catalyse the exchange of a H2A-H2B dimer (Bruno *et al*. 2003). However, only one remodelling complex, the SWR1 complex, was shown up to now to facilitate incorporation of H2A.Z into nucleosomes (Kobor *et al*. 2004; Mizuguchi *et al*. 2004).

Remodelling by the SWR1 complex enables DNA translocation by pumping DNA towards the histone octamer. This results in the formation of a DNA wave on the nucleosomal surface, which leads to a break of histone/DNA interactions, especially the one of H2A-H2B dimers. SWR1 was shown to remove the loosen H2A-H2B dimer and replace it with H2A.Z-H2B (Mizuguchi *et al*. 2004). The H2A.Z variant actually plays a role in repressing the spreading of heterochromatin at telomeres and is also a mark for poised promoters (see chapter on histone variants). Additionally, SWR1 shares some members with the HAT complex NuA4, suggesting a link of H2A.Z replacement and histone acetylation (Keogh *et al*. 2006).

Remodelling by RNAP II

In addition to ATP-dependent chromatin remodelling complexes reorganisation of chromatin can also be achieved by the traversing RNA polymerase itself (Studitsky *et al*. 2004).

Genes transcribed by RNA polymerase III are covered with transcription factors to prevent the formation of nucleosomes, thus chromatin remodelling is not required for transcriptional ignition or elongation. In contrast, RNA polymerase I transcribed genes for rRNAs and snRNAs contain nucleosomes, which are disrupted by an unknown RNAP I dependent remodelling mechanism. RNA polymerase II transcribed genes usually retain nucleosomal structure but have to undergo extensive chromatin remodelling to enable transcription. ATP-dependent remodelling complexes facilitate transcription by mechanisms, which involve nucleosome disruption, eviction and reassembly as described above. Another factor referred to as "facilitates chromatin transcription" (FACT) stimulates transcription through chromatin independently of ATP. FACT possesses an intrinsic chaperone character and disassembles H2A-H2B dimers from nucleosomes (Belotserkovskaya *et al*. 2003). It was shown that FACT is not only responsible for nucleosome disruption, but is also required for proper nucleosome reassembly behind the elongating polymerase (Kaplan *et al*. 2003). Another factor that might be involved in the remodelling of nucleosomes during transcription is the elongation factor SPT6, which possesses H3-H4 histone chaperone activity (Bortvin and Winston 1996; Kaplan *et al*. 2003).

HISTONE VARIANTS IN ACTIVE CHROMATIN

In addition to post-translational modifications of histones, histone variants play an important role as epigenetic marks. Different variants of histones H3 and H2A distinguish alternative chromatin states and participate in the assembly of specialised chromatin structures like centromere or telomeres and formation of the inactive X chromosome in female mammals. Importantly, the presence of some histone variants is closely linked to actively transcribed loci. The so-called replicative variants (H1, H2A, H2B, H3 and H4) are synthesized and incorporated only during the S phase, whereas the replacement variants are expressed constitutively throughout the cell cycle.

Although there are several mammalian histone variants, only two are common for all eukaryotes. All organisms express an evolutionary conserved, centromere-specific histone H3 variant (CenH3 or Centromere protein A (CENP-A)), which is critical for proper centromere function and the histone H2A variant referred to as H2A.Z in mammals and Htz1 in *S. cerevisiae*.

Besides the centromere-specific variant, in mammals histone H3 exists as two replicative variants (H3.1 and H3.2) and one replacement variant H3.3. Whereas the former two are exclusively synthesised and incorporated into chromatin during S phase, H3.3 can be integrated throughout the cell cycle by a replication-independent mechanism and is a mark of actively transcribed chromatin (Ahmad and Henikoff 2002).

H2A.Z, a H2A histone variant with only about 60% identity with the canonical H2A isoform, is also associated with active chromatin. In budding yeast, H2A.Z is found near silenced regions, like telomeres, where it inhibits the spreading of silent heterochromatin (Meneghini *et al*. 2003). Besides H2A.Z, other H2A variants are associated with diverse chromatic states. While macroH2A is enriched on the human inactive X chromosome, especially at regions that undergo transcriptional silencing (Chadwick and Willard 2002), H2A-Bbd (Barr-body-deficient), is widely distributed with the exception of the inactive X chromosome (the Barr body), suggesting that this pair of H2A variants mark alternative epigenetic states (Chadwick and Willard 2001). H2A.X is assembled at sites of double-strand brakes and phosphorylation of its C-terminal motif is an important event for recruitment of DNA-repair machineries (Fernandez-Capetillo *et al*. 2004).

The histone H2A.Z variant

H2A.Z was shown to be required for full expression of about 200 genes in yeast, suggesting a role of H2A.Z replacement in gene activation (Meneghini *et al*. 2003). Originally, H2A.Z was identified as a factor that limited the spreading of telomeric heterochromatin and was therefore described as an "antisilencing barrier" (Dryhurst *et al*. 2004). Importantly, histone acetylation, especially of K16 on histone H4, was found to be of tremendous importance for this function. In mammals, in contrast, H2A.Z is also enriched in heterochromatic foci together with HP1 and knock down experiments of this variant resulted in genomic instability, suggesting that H2A.Z might have diverse functions in different organisms (Fan *et al*. 2004).

Genome-wide studies in budding yeast revealed a number of RNAP II promoters, which are enriched for the Htz1 variant (the yeast homologue of H2A.Z in mammals) (Zhang *et al*. 2005). The SWR1 complex, closely related to the SWI/SNF family of ATP-dependent nucleosome remodelling enzymes, was found to be responsible for the exchange of H2A.Z-H2B and H2A-H2B dimers (Mizuguchi *et al*. 2004).

Htz1 localizes preferentially at TATA-less promoters of repressed genes and correlates with particular histone acetylation patterns (H3K14ac and H4K8ac). Nucleosomes containing Htz1 were shown to be less stable than H2A nucleosomes and are lost upon gene activation. Whether the entire nucleosome or just the Htz1-H2B dimers are ejected is still to be evaluated. Taken together, Htz1 variant is a general activator that facilitates induction of gene expression through its susceptibility to loss from nucleosomes (Zhang *et al*. 2005).

Another factor that mediates histone exchange is FACT, a histone chaperone that selectively removes H2A-H2B dimers, thereby destabilising the nucleosome during transcription elongation (Kaplan *et al*. 2003). A further histone chaperone, the nucleosome assembly protein 1 (NAP-1) is involved in removing and replacing H2A-H2B dimers, resulting in nucleosome sliding and histone exchange. This process is independent of ATP, but the kinetics of these reactions is very slow (Park *et al*. 2005).

The histone H3.3 variant

Histone H3.3 differs from canonical H3.1 and H3.2 at only five or four amino acid positions, respectively (**Fig. 5**). Whereas H3.1 and H3.2 variants are expressed in S-phase, the H3.3 variant is constitutively expressed and is the dominant H3 isoform in differentiated cells. Thus, H3.3 is also referred to as a canonical histone version. While H3.1 assembly into chromatin during replication is regulated by a replication-specific complex that includes the chromatin assembly factor 1 (CAF1), H3.3 deposition occurs by a replication-independent mechanism and involves a specialised chaperone complex containing the histone regulator A (HIRA) (Ahmad *et al*. 2002; Henikoff and Ahmad 2005). Importantly, H3.3 containing nucleosomes exclusively contain this particular H3 variant and not H3.1 or H3.2 (Loyola *et al*. 2006).

Up to now, it is not clear why the cell distinguishes between the replication and the replacement variant, as either of these variants lead to almost identical nucleosomal structures. The only divergent amino acid in the amino-terminal tail of H3.3 is H3.3S31 that can be phosphorylated during mitosis (Hake *et al*. 2005). However, this phosphorylation is distinct from other mitotic marks, such as H3S10ph and H3S28ph in histone H3 as it is present only in late prometaphase and metaphase around centromeres. Other differences in the H3.3 amino acid composition are located within the globular domain. Three unique

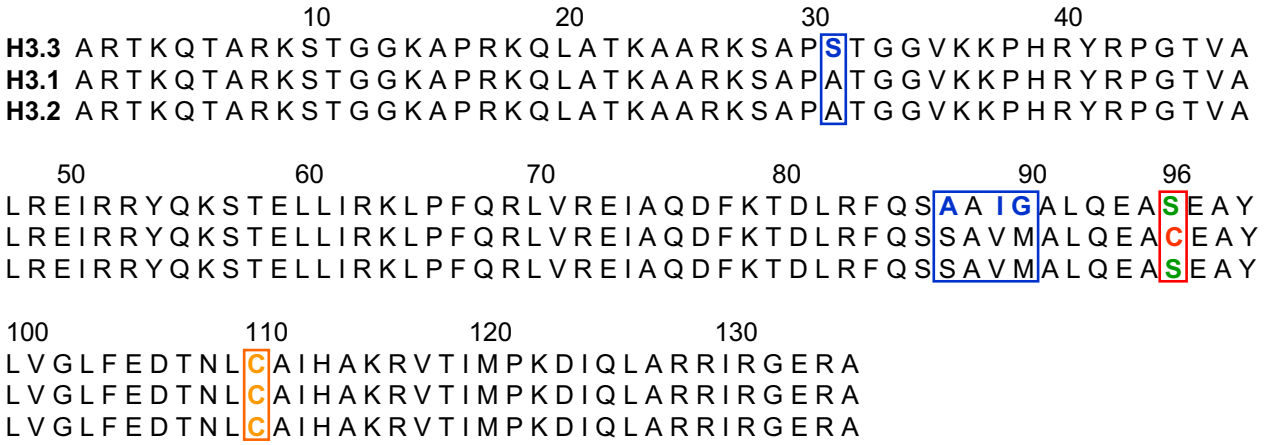

Fig. 5 Alignment of histone H3 variants. The three canonical histone H3 variants, H3.1, H3.2 and H3.3 differ only in a few but critical amino acids. H3.3 contains a serine at position 31, which is phosphorylated during mitosis. Three alterations at position 87, 89 and 90 were found to be sufficient for replication-independent incorporation of H3.3 via the HIRA complex. All three histone variants possess a cysteine at position 110, which is thought to form internucleosomal disulfide bonds. H3.1 contains a unique cysteine at position 96, which constitutes the sole difference between H3.1 and H3.2. This residue may be involved in the formation of intramolecular disulfide bonds (details see text).

amino acids (positions 87, 89 and 90) within histone H3.3 that further distinguish this variant from H3.1 and H3.2 were found to be crucial for the replication-independent incorporation via the HIRA complex (Ahmad *et al*. 2002). Another difference between H3.3 and H3.1 concerns position 96. Whereas H3.2 and H3.3 contain a serine at this position H3.1, contains a cysteine at this site. The other cysteine at position 110 is common for all H3 variant and it was recognised quite early that this residue is masked and inaccessible in transcriptionally silent chromatin (Allfrey *et al*. 1964). Further it was suggested, that H3C110 forms intramolecular disulfide bonds, within the nucleosome, corresponding to a reversible transcriptional repressive state, whereas H3.1C96 forms intermolecular disulfide bonds to other nucleosomes or components of the nuclear envelope such as the lamin B receptor (LBR), leading to the formation of transcriptional repressive heterochromatin (Hake and Allis 2006a).

Strikingly, also the PTM patterns observed on the different H3 variants are different. H3.3 is particular enriched in modifications associated with transcriptional active chromatin, but depleted for repressive modifications like H3K9 methylation, affirming studies that demonstrated H3.3 incorporation during transcription. In contrast H3.1 and H3.2 were found to carry mainly repressive modification patterns (McKittrick *et al*. 2004; Hake *et al*. 2006b).

Genome-wide studies in *Drosophila* confirmed that H3.3 replacement occurs at actively transcribed genes in correlation with abundant RNAP II (Mito *et al*. 2005). In addition global profiling of H3.3 incorporation in *Drosophila* SL2 cells showed that even though promoters of active genes were partly depleted of histones, H3.3 was predominantly found at the very proximal promoter regions and within coding regions with a decline from 5′ to 3′ (Mito *et al*. 2005). In contrast, another study in *Drosophila* Kc cells revealed that H3.3 was absent of promoters and was found only throughout transcribed regions (Wirbelauer *et al*. 2005).

In mammals, H3.3 replacement seems to differ from the fly system. In mouse fibroblasts, H3.3 deposition correlated with transcription of E2F regulated genes. However, incorporation was observed predominantly at promoters, whereas downstream regions showed lower levels of H3.3 (Daury *et al*. 2006). Another study in mouse pro- and pre-B-cells showed H3.3 replacement mainly upstream from the transcription initiation site of active genes (Chow *et al*. 2005). Therefore it was suggested that H3.3 incorporation in mammals is rather associated with chromatin remodelling and promoter function and less linked to transcriptional elongation.

In yeast, the situation was found to be completely different. Whereas *S. pombe* expresses one H3 variant with hallmarks of H3.3 and H3.2, H3.3 is the only variant found in *S. cerevisiae*, fitting to the model of the "active genome" of this organism and the lack of transcriptional repressive H3K9 methylation. During transcription $(H3\text{-}H4)_2$ tetramers are removed before RNAP II is traversing and are then rapidly reloaded *in trans* (Schwabish and Struhl 2004). Therefore it will be an important task to further address the differences in histone variant incorporation between different organisms.

Chromatin assembly

It is believed that $(H3\text{-}H4)_2$ tetramers, comprising specific modification patterns (especially acetylation), build the first contact to DNA. However, the deposition complexes CAF1 and HIRA were shown to be associated with H3-H4 dimers and not tetramers (Tagami *et al*. 2004). Thus, H3 and H4 are deposited as dimers rather than tetramers. CAF1 associates specifically with H3.1-H4 dimers and is involved in a nucleosome assembly pathway dependent on DNA synthesis (replication-coupled (RC) pathway), whereas HIRA binds H3.3-H4 dimers and was shown to mediate a nucleosome assembly pathway independent of replication (replication-independent (RI) pathway) (Tagami *et al*. 2004). Within either complex the anti-silencing function 1 protein (ASF1), a member of the N1 chaperone family, is also present together with dimeric forms of H3 and H4. Once the tetramer is formed on the DNA, nucleosome assembly protein-1 (NAP-1), an additional histone chaperone of the nucleoplasmin family, adds the H2A-H2B dimers to complete the nucleosome structure. Finally, acetyl moieties are removed from the newly assembled chromatin by HDACs (Polo and Almouzni 2006).

Currently, there are two models how chromatin is assembled especially during replication. According to the conservative model newly replicated DNA is assembled into chromatin using *de novo* synthesised histones and old parental histones into separate ways. At the replication folk, the parental nucleosome is suggested to be transiently disrupted into two H2A-H2B dimers and one $(H3\text{-}H4)_2$ tetramer. The tetramer either stays on the leading strand or is transferred to the newly replicated strand randomly. The newly synthesised tetramer is assembled on the other daughter strand in a CAF1-dependent manner. According to this model old and new histones are not found in the same nucleosomes.

The semi-conservative assembly model suggests that parental and *de-novo* synthesised histones are assembled

into the same nucleosome. $(H3\text{-}H4)_2$ tetramers are split into two heterodimers, which are equally distributed to the two daughter strands and then are paired with newly synthesised H3-H4 dimers by CAF1 (Tagami *et al*. 2004).

Up to now, it is not clear according to which mechanism chromatin is assembled *in vivo*. The semi-conservative model provides an elegant mechanism for the inheritance of epigenetic chromatin states, as the chromatin composition is identical in both daughter strands after replication. The fact that nucleosomes are homogenous in their H3 composition (Loyola *et al*. 2006) either implicates a mechanism to convert mixed (H3.1/H3.3) tetramers into homogenous (H3.1/H3.1) tetramers or renders the conservative model to be more likely.

OUTLOOK

It is of enormous importance to understand the mechanisms that regulate transcription, DNA repair and other biological issues by modifying chromatin structures. Until recently the study of human diseases has focused on genetic changes on the DNA level, while it is becoming increasingly clear that a number of disorders are linked to epigenetic and often heritable alterations. Aberrant DNA methylation and changes in histone PTM patterns are associated with various disorders such as cancer or autoimmune diseases.

In this review we discussed three different mechanisms that are required for the modulation of chromatin architecture: the PTMs of histones, ATP-dependent chromatin remodelling and the incorporation of specialised histone variants. All these events are well established as important factors for transcriptional activation or repression. Misleadingly, they are frequently looked at as separate and isolated mechanisms. However, the examples discussed above clearly point out that all three mechanisms are connected events although they may be of different relevance at particular genomic regions. Because of this crosstalk it is not surprising that enzymes involved in all three mechanisms are often found together in multi-protein complexes.

A reciprocal interaction may be most obvious for histone PTMs and examples of an interplay between different modifications *in cis* and *in trans* are well established. Indeed various proteins beside histones are known to undergo multiple PTMs in an interdependent fashion. As mentioned above, the basis for such modification crosstalk may be the assembly of several enzymatic activities into multiprotein complexes, the direct influence of "early" or stable modifications on the recruitment or enzymatic activity of "delayed" modifiers, or the impact of PTMs on protein-protein interactions. Therefore, an important task for future research will be to address the modification "make-up" of histones (and other proteins) during different cellular conditions and the effects of such combinatorial modifications. One approach would be the use of antibodies directed against multiple modified histone molecules, such as phosphoacetylation or phosphomethylation. Mass spectrometry and proteomic methods constitute another promising approach for the generation of histone "modification maps", complementing modification specific antibodies without the drawback of the sometimes unreliable epitope specificity. However, for mapping of modification patterns to particular genomic regions, methods like ChIP-on-chip assays are still indispensable, due to the inability to enrich certain genomic regions in a way that would allow mass spectrometric analysis of the associated chromatin components.

The identification of modification-dependent interaction domains, such as the chromo- or bromodomains, provided the basis for the analysis of the biological effects of PTMs on protein-protein interactions. Recent observations point to further layer of complexity; as such interactions may be modulated by PTM combinations, exemplified by the mitotic "binary-switching" demonstrated for HP1 proteins (Mateescu *et al*. 2004; Fischle *et al*. 2005; Hirota *et al*. 2005). Indeed modification-dependent protein interactions under certain conditions may also provide the basis for the targeting further enzymatic activities like chromatin remodellers, as has been discussed above.

The importance of histone PTMs in the formation and also the propagation of particular chromatic states are also emphasised by the observation that different histone H3 variants are differentially enriched for PTMs either associated with transcriptional active or repressive chromatin. These specialised histone variants were suggested to constitute an "index" or genomic "barcode" (Hake *et al*. 2006a), which would also emphasise the differential PTMs on different variants.

Concluding, the formation and interpretation of transcriptionally active chromatin involves multiple mechanisms at several different levels that collaborate on the constitution of a particular but dynamic epigenetic state.

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