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Review

Histone H3 phosphorylation – A versatile chromatin modification for different occasions

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ABSTRACT

Post-translation modifications of histones modulate the accessibility and transcriptional competence of specific chromatin regions within the eukaryotic genome. Phosphorylation of histone H3 is unique in the sense that it associates on one hand with open chromatin during gene activation and marks on the other hand highly condensed chromatin during mitosis. Phosphorylation of serine residues at histone H3 is a highly dynamic process that creates together with acetylation and methylation marks at neighboring lysine residues specific combinatorial patterns that are read by specific detector proteins. In this review we describe the importance of different histone H3 phosphorylation marks for chromatin condensation during mitosis. In addition, we review the signals that trigger histone H3 phosphorylation and the factors that control this reversible modification during interphase and mediate the biological readout of the signal. Finally, we discuss different models describing the role of histone H3 phosphorylation in the activation of transcription of poised genes or by transient derepression of epigenetically silenced genes. We propose that histone H3 phosphorylation in the context with lysine methylation might temporarily relieve the silencing of specific genes without affecting the epigenetic memory.

1. Introduction

Eukaryotic DNA associates with histones and other proteins, forming the stable but dynamic structure of chromatin. Histones are among the most highly evolutionary conserved proteins and build as octamers together with approximately 147 base pairs of

histone fold that mediates the interactions between histones within the nucleosome, as a well as a flexible N-terminal tail, protruding from the nucleosomal core [2]. The N-terminal tails of histones, as well as their globular domains, are subjected to a large variety of reversible covalent posttranslational modifications (PTMs), such as acetylation, methylation, phosphorylation, and ADP-ribosylation, which are implicated in the regulation of a multitude of biological processes, including transcription [3]. PTMs of histones can not only alter the biophysical properties of the interactions between histones and DNA but also affect the binding of a variety of effector proteins that mediate further biological events. As a result, they can either overcome an inherently repressive nature of chromatin to establish a transcriptionally permissive state or participate in the silencing of a given gene [3,4].

It has been proposed that combination of differentially modified histone tails constitutes a code that predicts a status of a chromatin region [5]. Indeed, genome-wide studies employing chromatin immunoprecipitation (ChIP) and state-of-the-art high throughput ations of genomic elements [6–9]. However, to provide relevant insights into understanding a function of a particular histone PTM, those patterns need to be interpreted in the context of other epigenetic and transcriptional regulators, taking into consideration the physiological state of the cell. This requirement stems from the fact that chromatin does not only provide a structural scaffold but is an essential regulatory component of a signaling network that integrates the incoming signals and dictates biological output [10–12]. However, in contrast to any other cellular signaling pathway, chromatin provides a unique possibility to maintain established patterns of PTMs through cell divisions in the absence of initial signal, what enables the inheritance of cell type specific gene expression patterns established during cell lineage specification and thus preserves cellular identity [11]. Yet, some of the histone marks appear only transiently to modulate a chromatin landscape as an intermediate state in a multistage process or as a consequence of a cellular response to changes in extracellular environment [13].

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Direct phosphorylation of specific residues of histone H3 at inducible promoters during interphase by signal transduction kinases, concomitant with rapid transcriptional induction of those genes, provides probably the best example of such a dynamic chromatin marking [14,15]. Interestingly enough, very well documented phosphorylation of the same residues during mitosis correlates with chromatin condensation and transcriptional repression [16], supporting the rationale for context-dependent interpretation of chromatin modifications. In this review we summarize the current knowledge on the role of histone H3 phosphorylation in chromatin biology of mammalian cells with the major focus on mechanistic aspects underlying its function in such complex process as cellular response to stress and mitogenic stimulation, immune response, memory formation and neuronal plasticity.

2. Histone H3 phosphorylation during mitosis

Phosphorylation of histone H3 has gained a considerable attention since it was demonstrated to closely associate with condensation of mitotic chromosomes in mammalian cells [17,18]. Although this correlation has been observed during mitosis and meiosis in a wide range of eukaryotes, the functional significance of this modification at individual sites of histone H3 differs among species [19–24]. Mitotic histone H3 phosphorylation in mammalian cells occurs massively at several residues, including serines 10 and 28 as well as threonines 3 and 11 and exhibits highly coordinated spatio-temporal distribution [25–27]. The development of antibodies specifically recognizing histone marks has enabled to precisely determine the kinetics of phosphorylation events at individual sites. However, those results need to be interpreted with caution bearing in mind a possible problem of antibody occlusion resulting from a negative impact of densely distributed multiple modifications, which has been reported for several antibodies widely used in the chromatin field [26,28].

The phosphorylation target sites at histone H3, H3S10 and H3S28 are each part of the identical sequence motif –ARKS- (Fig. 1) and show only subtle differences in their temporal modification patterns during mitosis. In mammalian cells, phosphorylation at those sites is mediated by Aurora-B kinase and initiates at pericentromeric heterochromatin regions at the onset of mitosis and then spreads along the entire length of chromosomal arms to reach the maximal abundance during metaphase, followed by a rapid decrease upon transition to anaphase [16,29]. This characteristic pattern, highly coincident with chromosomal condensation both in space and time, indicates a potential role of site-specific histone H3 phosphorylation in this process [19].

However, a causal relationship between those two phenomena can not be easily determined by genetic means due to the presence of multiple copies of histone H3 gene in higher eukaryotes what imposes a formidable obstacle to directly address the role of individual modifications by mutation of specific phosphorylation sites. Therefore, lower eukaryotes provide an excellent model to study a causal link between histone phosphorylation and chromosome dynamics *in vivo*. In line with that, *S. pombe* mutants carrying an S10A mutation display numerous mitotic defects, including impaired chromosome segregation [30]. Interestingly, in *S. cerevisiae* H3S10ph and H3S28ph marks seem to be dispensable for cell cycle progression as mutations of individual residues (S10A, S28A) as well as the double mutation S10AS28A affect neither mitotic nor meiotic chromosome transmission, however in this organism H2B phosphorylation may have a redundant role, since Ipl1p kinase – the yeast ortholog of Aurora-B - is able to phosphorylate histone H2B in a mixture of free histones [20]. In contrast, H3S10ph is absolutely required for proper execution of mitosis and meiosis in *Tetrahymena*, as S10A mutation results in chromosome condensation and segregation defects, leading to chromosome loss [24]. Precise spatio-temporal patterns of mitotic phosphorylation at H3S10 and H3S28 indicate the existence of mechanisms that tightly control the activity and localization of mitotic kinases. Indeed, only recently it has been shown that Haspin-mediated phosphorylation of H3T3 is crucial for the recruitment of Aurora-B to centromeres and its subsequent activation [31,32]. However, Haspin RNAi does not affect the levels of H3S10ph indicating that not all mitotic functions of Aurora-B depend on this interaction. Interestingly, although temporal pattern of H3T3ph during mitosis closely resembles the one of H3S10ph, localizations of the two marks only partially overlap, as H3T3ph originates at chromosomal arms in late G2 and during metaphase becomes intensively concentrated at inner centromeres, consistent with its role in regulating centromeric functions of Aurora-B-containing chromosome passenger complex [31–33]. Finally, mitosis-specific phosphorylation of H3T11, resulting from Dlk/ZIP kinase activity, appears to be restricted to the centromeric regions during metaphase, where it was proposed to play a role for kinetochore assembly [9,34].

3. Interphase phosphorylation of histone H3

3.1. The nucleosomal response

The activation and orchestrated action of signaling kinases enables adaptive responses to a wide variety of extracellular stimuli by the initiation of specific transcription programs. Therefore, the transfer *via* a phosphorylation cascade could constitute an efficient and rapid mechanism to deliver a signal registered at the plasma membrane to the nucleus. Indeed, a growing body of evidence indicates that the action of kinases is not restricted to the cytoplasmic compartment as they are able to translocate to the nucleus

Fig. 1. Sites of histone H3 tail modifications. Target residues for phosphorylation (blue), lysine methylation (yellow) and acetylation (red) within the N-terminal region of histone H3 are shown. The ARKS modification boxes are highlighted by an orange frame.

to phosphorylate their substrates, including transcription factors, chromatin-modifying complexes and components of the transcriptional machinery [35,36]. Furthermore, signaling kinases were found to stably associate with promoters as well as gene body regions to regulate the activity of those elements [37–39]. Early studies in PC12 cells treated with epidermal growth factor (EGF) provided the first indication that histone H3 could be a substrate for a signaling kinase [40]. Further evidence supporting this hypothesis arose from identification of the nucleosomal response. This term describes the rapid and transient phosphorylation of histone H3 at serine 10 and HMG-14 at serine 6, that accompanies the transcriptional activation of immediate early genes *c-fos* and *c-jun* in quiescent fibroblasts stimulated with growth factors, phorbol esters, as well as phosphatase and protein synthesis inhibitors [41]. In contrast to global mitotic phosphorylation of histone H3, the stimulus-induced H3 phosphorylation targeted only a minute fraction of nucleosomes [42]. Moreover, the kinetics of histone H3 phosphorylation upon stimulation with abovementioned agents closely paralleled the expression profiles of induced genes, suggesting a link between those two processes. Importantly, the presence of transcriptional inhibitors actinomycin D and α-amanitin did not abolish histone H3 phosphorylation in response to the stimuli, excluding the possibility that this modification is a consequence of gene activation [41]. Further studies using the kinase inhibitor H89 demonstrated that the nucleosomal response is mediated, depending on the stimulus, either *via* the extracellular signal – regulated kinase (ERK) or p38 MAP kinase cascades, but not the c-Jun amino-terminal kinase/stress-activated kinase (JNK/SAPK) pathway [43].

3.2. The effector kinases

Given that the motif containing H3S10 does not resemble the consensus sequence for ERK and p38, the effector kinases downstream of MAPK activation emerged as putative candidates for targeting histone H3. In particular, mitogen- and stress-activated kinase 1 (MSK1) as well as ribosomal subunit protein S6 kinase 2 (RSK2) were taken into consideration, due to their nuclear localization upon signaling and the ability to phosphorylate histone H3 peptide *in vitro*. Direct comparison of MSK1 and RSK2 activities towards histone H3 as a substrate determined by *in vitro* kinase assays revealed 4-fold higher efficiency of MSK1. In addition, MSK1 but not RSK2 was sensitive to H89 in concentration that inhibited nucleosomal response *in vivo*, leading to the conclusion that MSK1 is potential kinase implicated in phosphorylating histone H3 [43]. However, the analysis of nucleosomal response in fibroblast isolated from a patient suffering from Coffin-Lowry syndrome (CLS), a disease associated with mutations in *RSK2* gene, suggested that RSK2 is required for EGF induced phosphorylation of histone H3 [44]. Upon EGF stimulation, phosphorylation of histone H3 in CLS fibroblasts was impaired, although mitotic histone H3 phosphorylation remained intact. Moreover, reconstitution of RSK2 activity by ectopic expression of wild-type RSK2 restored EGF induced phosphorylation of histone H3 in those cells. In addition, disruption of the *Rsk2* gene in mouse embryonic cells by homologous recombination resulted in a decreased level of histone H3 phosphorylation upon EGF treatment indicating that RSK2 is a principal kinase responsible for targeting histone H3 in response to EGF stimulation [44]. However, a more recent study failed to reproduce these results, as histone H3 phosphorylation in response to cellular stress or mitogenic stimulation in CLS cells originating from the same source was not affected [15]. The reason for the apparent discrepancy between the two studies still remains unclear. Furthermore, fibroblasts derived from MSK1 and MSK2 deficient mice showed severely impaired H3 phosphorylation upon anisomycin and 12-O tetradecanoylphorbol-13-acetate (TPA) stimulation, accompanied by reduced activation of immediate early genes [15], in agreement with previous reports on the predominant role of MSKs in targeting histone H3 during nucleosomal response [43]. Interestingly, although [^{32}P]-labeling of living fibroblasts demonstrated a slight reduction in anisomycin stimulated H3S10 phosphorylation in MSK1 deficient cells, the response was completely diminished in MSK2 and MSK1/2 double-knockout fibroblasts, suggesting that MSK2 is a major kinase mediating nucleosomal response in those cells in response to stress inducer.

Moreover, anisomycin and TPA induced phosphorylation of another site at histone H3, serine 28, which had been previously described as a MSK1 target upon ultraviolet B (UVB) irradiation of JB6 mouse epidermal cells [15,45]. In agreement with that, H3S28 phosphorylation was also affected by MSK1/MSK2 ablation, although to a lesser extent than H3S10 [15].

3.3. Cell type specific function of histone H3 phosphorylation

Interestingly, H-*Ras* transformed fibroblasts exhibited higher steady-state levels of H3 phosphorylation at both sites in comparison to non-tumorigenic parental strain as a consequence of constitutive activation of ERK/MAP kinase pathway [46]. Since somatic gain-of-function mutations in *RAS* genes were reported to occur in nearly 30% of human cancers [47], MSK1 mediated histone H3 phosphorylation downstream hyperactive Ras presumably contributes to neoplastic transformation. Indeed, siRNA mediated knockdown of MSK1 as well as its dominant-negative mutant suppressed TPA and EGF induced transformation of JB6 mouse epidermal skin cells, indicating a crucial role of MSK1 in tumorigenesis [48]. In line with that, ChIP studies in *Ras* transformed fibroblasts revealed that elevated expression of IE gene products JUN, FRA-1 and COX-2 was accompanied by increased levels of H3S10 and H3S28 phosphorylation at their promoters [49]. This effect was shown to be MSK1 dependent as reducing the kinase activity either by chemical inhibition or knockdown resulted in a significant decrease in expression of abovementioned genes and subsequent loss of the malignant phenotype, suggesting a significant contribution of MSK1 and maybe also the nucleosomal response to tumorigenic potential of malignant cells [49].

Ubiquitous expression of MSKs in mouse and human suggests their participation in a large variety of cell type specific responses [50]. Interestingly, MSK1 and 2 transcript levels were shown to be markedly enriched in the brain, in contrast to RSK2, whose mRNA was only lowly expressed in this organ [50]. Detailed expression pattern analysis by immunohistochemistry in adult mouse brain revealed that MSK1 is mainly present in striatal and olfactory tubercle neurons as well as in hippocampus, exhibiting nuclear localization in all expressing cells, consistent with its function as histone H3 kinase [51,52]. This observation suggested that nucleosomal response contributes to ERK/MAPK mediated transient induction IE genes critical for neuronal plasticity in those brain regions. Strikingly, histone H3 phosphorylation was demonstrated to accompany ERK/MAPK pathway mediated activation of *c-Fos* by agonists of dopamine (DA), muscarinic acetylcholine (mACh) and glutamate (GLU) receptor in hippocampal neurons, suggesting an important role of nucleosomal response in memory formation [53]. Supporting this hypothesis, MSK1 deficient mice, despite no apparent changes in overall brain morphology, displayed numerous behavioral defects, including impaired Pavlovian fear conditioning and spatial learning, what was attributed to the failure in inducing histone H3 and c-AMP response-element-binding protein (CREB) phosphorylation downstream ERK/MAPK pathway in hippocampal neurons [51,52]. In addition, mice lacking MSK1 showed decreased locomotor sensitization in response to repeated cocaine

administration due to the failure to induce c-fos and dynorphin in their striatal neurons [54]. In conclusion, MSK1 emerges as a crucial regulator of neuronal functions upon stimulation with extracellular signals as its deficiency results in severe behavioral abnormalities. It remains a major challenge to determine to which extent histone H3 phosphorylation contributes to this regulation.

3.4. Other histone H3 kinases

Of note, although the best studied, MSKs are not the sole kinases capable of targeting histone H3S10 during interphase (see Table 1). It was shown that IKK-α is responsible for cytokine induced H3S10 phosphorylation at the promoters of NF-κB responsive genes, IκBα and IL-8, in mouse embryonic fibroblasts [55,56]. Interestingly, the induction of a subset of NF-κB target genes following LPS treatment is accompanied by H3S10 phosphorylation in a p38-dependent manner in primary human monocyte-derived dendritic cells [57]. These data indicate a potential role of histone phosphorylation in activating inducible transcription programs in the immune system.

Moreover, neuronal stem cell differentiation was demonstrated to require chromatin-modifying activity of JNK [39]. This MAP kinase was shown to phosphorylate histone H3 at serine 10 at the promoters of genes critical for developmental processes [39]. In addition, c-AMP dependent protein-kinase-A (PKA) was reported to target H3S10 upon follicle stimulated hormone (FSH) stimulation in immature ovarian granulosa cells promoting their differentiation, what further suggests that this histone modification participates in regulation of transcription during development [58]. Strikingly, the exposure of JB6 mouse epidermal cells to the common environmental carcinogen arsenite resulted in Akt1 dependent phosphorylation of H3S10 [59]. Interestingly enough, Cot kinase was shown to modify this site as well, what was required for upregulation of c-*Fos* gene and neoplastic transformation [60]. Moreover, H3S10 was demonstrated to be a substrate for PIM1 kinase, implicated in regulation of 20% of c-Myc target genes and thereby contributing to their transcriptional activation, what further links this PTM of histone H3 to cellular transformation [61].

Table 1
Regulators of histone H3 phosphorylation.

Regulators	Histone mark	References
WRITERS		
Aurora-B	H3S10, H3S28	[20] [33],
Haspin	H3T3	[35] [36],
Dlk/ZIP	H3T11	[13] [38],
MSK1/2	H3S10, H3S28	[19] [47], [49] [52], [53]
IKK-α	H3S10	[59] [60],
JNK	H3S10	[43]
PKA	H3S10	[62]
Akt	H3S10	[63]
Cot	H3S10	[64]
PIM1	H3S10	[65] [87],
CDK8	H3S10	[89]
JAK2	H3Y41	[66]
PKCβ	H3T6	[67]
PKCδ	H3T45	[69]
Chk1	H3T11	[70]
Positively affected READERS		
14-3-3	H3S10	[82–84]
Survivin	H3T3	[35] [36],
JMJD2C	H3T11	[68]
Negatively affected READERS		
HP1α, β and γ	H3S10	[84] [91], [92]
PcG	H3S28	[95] [96],
TFIID	H3T3	[93]
LSD1	H3T6	[67]
ERASERS		
PP1	H3T3, H3T11, H3S10, H3S28	[98]
PP2A	H3S10	[80]

3.5. Additional histone H3 phosphorylation sites

It should be noted that in addition to serine 10 and serine 28, N-terminal tail of histone H3 bears many more sites that are phosphorylated by different signaling kinases in response to extracellular signaling. JAK2 was shown to phosphorylate H3Y41 what resulted in transcriptional activation of its target genes, including hematopoietic oncogene *Imo2* [62]. In addition, androgen receptor dependent gene activation was demonstrated to implicate the phosphorylation of H3T6 by protein-kinase-C beta (PKCβ) [63], as well as protein-kinase-C-related kinase 1 (PRK1) mediated phosphorylation of H3T11 [64]. Moreover, apoptotic events in neutrophils were shown to be accompanied by H3T45 phosphorylation catalyzed by protein-kinase-C delta (PKCδ), constituting an interesting example for the role of histone H3 phosphorylation in programmed cell death [65]. In addition, H3T3 phosphorylation by checkpoint kinase 1 (Chk1) kinase was reported to confer transcriptional activity of *cyclin B1* and *cdk1* promoters [66]. Upon DNA damage, Chk1 became released from its target promoters, leading to dephosphorylation of H3T11 and subsequent transcriptional repression [66], further expanding the large repertoire of the functions of histone H3 phosphorylation to the regulation of the cell cycle.

Taken together, an increasing body of evidence indicates that site-specific phosphorylation of histone H3 mediates the readout of a large variety of extracellular signaling pathways by regulating gene expression patterns. As the field of histone phosphorylation is rapidly developing, future studies will probably identify a lot more kinases that target histone H3, what will enable a better understanding of cell type specific signaling events.

4. Mechanistic aspects of histone H3 phosphorylation

The most intriguing feature of histone H3 phosphorylation that makes it truly unique among all the other know histone PTMs is that it regulates two apparently opposite processes: condensation of mitotic chromosomes associated with transcriptionally repressive state as well as signal induced gene activation during interphase. Of note, the residues phosphorylated by mitotic kinases and the ones targeted during interphase by specific effector enzymes activated in response to extracellular signaling are essentially the same. Global histone H3 phosphorylation during mitosis is associated with chromatin compaction whereas stimulus-induced phosphorylation targets only a small fraction of histone H3 molecules, restricted to gene regulatory elements and confers the transcriptionally active state. The striking difference in the extent of histone H3 phosphorylation at those two stages could suggest that the relative amount of the mark within chromatin correlates with a given biological readout.

However, histone H3 phosphorylation in *Drosophila melanogaster* does not follow this rule as it targets euchromatic interband regions of polytene chromosomes on a global scale during interphase. This specific pattern is established by the JIL-1 tandem kinase, an ortholog of mammalian MSKs, which is essential for *Drosophila* viability [67]. Flies carrying *JIL-1* hypomorphic alleles are characterized by the absence of euchromatic interband regions, high condensation of chromosome arms and decreased levels of interphase but not mitotic H3S10ph. Interestingly, lethality of *JIL-1*-null mutants can be rescued by reduced activity of H3K9 methyltransferase Su(var)3–9, supporting the hypothesis that JIL-1 functions to counteract heterochromatic spreading [67,68]. In accordance with this idea, JIL-1 was identified as effector of position effect variegation (PEV) in the fly [69]. Thus, in *Drosophila*, in contrast to mammalian system, interphase phosphorylation of histone H3 plays a global role in maintaining the balance between euchromatin and heterochromatin.

An enormous diversity of processes regulated by histone H3 phosphorylation raises the question about the mechanistic aspects that underline the role of this modifications in a wide range of specific, and often opposite, cellular functions and over the last few years substantial progress has been made in identifying factors involved in translating histone H3 phosphorylation into biological outcomes.

Early studies on nucleosomal response in quiescent fibroblasts demonstrated that, in response to mitogen, histone H3 phosphorylation targeted the fraction of nucleosomes that was sensitive to sodium butyrate induced hyperacetylation, revealing an interesting link between those two modifications [42].

The development of dual specificity antibody, recognizing histone H3 when phosphorylated at serine 10 and simultaneously acetylated at lysine 14 enabled to address whether those two marks coexist at the histone H3 tail *in vivo*. Indeed, EGF stimulation resulted in accumulation of histones decorated with a dual mark (termed as histone H3 phosphoacetylation) [70]. Moreover, ChIP assays demonstrated the presence of phosphoacetylated histone H3 at *c-Fos* as well as *c-Jun* promoters upon MAP kinase pathway activation [14,70].

Two models explaining simultaneous targeting of phosphorylation and acetylation marks to the same histone H3 tail were proposed (Fig. 2). According to the first model, based on electrophoretic analysis of [^{32}P]-labeled histones in EGF and sodium butyrate treated fibroblasts as well as ChIP experiments using antibodies recognizing mutually exclusive subsets of nucleosomes, histone H3 phosphorylation and acetylation are two independent but spatially linked events and one modification is not a prerequisite for the other [42,71]. The alternative model implies that these two modifications are synergistic, as three yeast histone acetyltransferases Gcn5, PCAF and p300 exhibit higher activity towards lysine 14 of histone H3 peptides, which have been phosphorylated at serine 10 [70,72]. Moreover, upon mutation of arginine 164 of yeast Gnc5 [Gcn5(R164A)], a residue adjacent to H3S10 in the ternary structure of the Gcn5/CoA/histone H3 complex, Gcn5 activity towards phosphorylated peptide was reduced to the level when unmodified peptide was used as substrate. Strikingly, the same mutation of the enzyme reduced transcriptional activity of the same subset of promoters that had been shown to be affected by H3S10A substitution, demonstrating that acetylation of lysine 14 depends on previous phosphorylation of H3S10 *in vivo* [72]. However, elegant studies in yeast, using two genes regulated by H3S10ph and histone acetylation as an example, demonstrated that this effect is promoter specific [73,74]. In the case of *Gal1*, the Gal4 transcription factor recruited both the H3S10 kinase (Snf1) and the SAGA complex to the promoter. In contrast, the transcription factor Ino2 recruited Snf1 kinase but not acetyltransferase complex to the *INO1* promoter and H3K14 acetylation was dependent on Snf1 kinase activity. In conclusion, *Gal1* and *INO1* promoters required histone phosphorylation and acetylation for TBP recruitment and transcriptional activation. However, the recruitment of histone acetyltransferase was regulated by distinct mechanisms indicating that, depending on the promoter studied, both models explaining co-targeting of the two histone modifications to the same promoters are correct [73,74] (Fig. 2).

The results of several reports show that simultaneous induction of histone phosphorylation and acetylation are crucial for the activation of specific mammalian genes. For instance, it was shown that stable phosphoacetylation is required for transcriptional activation of the *HDAC1* gene, indicating that for some genes regulated by histone phosphorylation an additional acetylation signal is needed [75]. Interestingly, in quiescent fibroblasts stress-induced p21 expression is higher upon simultaneous HDAC inhibitor treatment, what is accompanied by higher levels of histone H3 phosphoacetylation at the *p21* promoter [76]. The same cooperative effect of the two marks was reported for a particular class of transposable elements, VL30 that undergo transcriptional activation upon stress induction, indicating that role regulatory of H3 phosphoacetylation is not restricted to protein coding genes [77].

Importantly, MSK1/2 mediated histone H3 phosphoacetylation resulting from stress or growth factor induced MAP kinase cascade is a transient event, at least in part due to chromatin associated activity of PP2A phosphatase, that was shown to dephosphorylate the H3S10ph [76]. Remarkably, simultaneous inhibition of histone deacetylase (HDAC) activity leads to the stabilization of the mark suggesting a mechanistic link between deacetylation and dephosphorylation.

The identification of the first, and so far only factor specifically recognizing H3S10ph *in vivo* – 14-3-3 – shed light on a function of signaling induced histone H3 phosphorylation in transcription [78–80]. Importantly, the motif containing serine 10 bound by 14-3-3 in histone H3 differs from two known consensus 14-3-3 binding motifs as it lacks strongly preferred proline residue at the position P+2. Consequently, *in vitro* peptide binding assays showed only moderate interaction of recombinant 14-3-3 with the H3S10ph peptide, which could be significantly enhanced by replacing glycine at position 12 with proline (H3S10phG12P) [81]. Strikingly, 14-3-3 binding to phosphoacetylated H3S10phK14ac peptide was as strong as to H3S10phG12P, indicating that acetylation of lysine 14 compensates for the lack of the proline residue in the histone H3 motif [81]. Therefore, a model has been proposed, where 14-3-3 binding to H3S10ph at target promoters is stabilized by additional acetylation of H3K9 or H3K14, thus protecting the phosphorylation mark from being removed by PP2A [76].

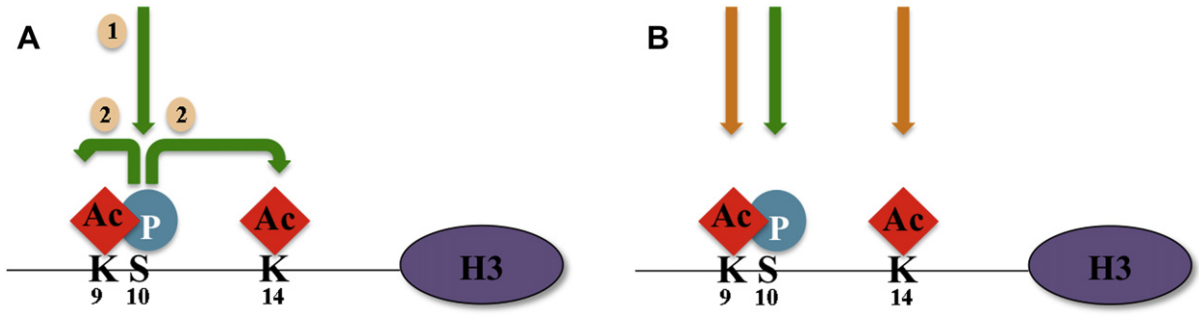

Fig. 2. Models for targeting of histone H3 by acetylation and phosphorylation. A. Model 1 – synergistic coupling of two events - phosphorylation of serine 10 (step 1) enables the recruitment of acetyltransferases (step 2), which then modify either H3K9 or H3K14. B. Model 2 – spatially linked but independent events – histone H3S10 phosphorylation targets hyperacetylated histone H3 but none of the modification is the prerequisite for the other.

In addition to shielding the H3S10ph mark, 14-3-3 constitutes a binding platform for factors involved in transcriptional regulation, thereby fulfilling its function as reader of a histone code. It was shown that upon MAP kinase signaling 14-3-3 recruited Brg1, the ATPase subunit of SWI/SNF remodeling complex to the histone H3-phosphorylated nucleosomes at the target promoters, what was followed by RNA polymerase II recruitment and subsequent transcriptional activation [82]. In addition, histone H3 phosphorylation was also demonstrated to regulate transcriptional elongation. In response to serum stimulation, PIM1 kinase-mediated phosphorylation of H3K9 acetylated nucleosomes at the *FosL1* enhancer was recognized by 14-3-3, which, in turn, recruited the histone acetyltransferase MOF. Upon recruitment, MOF catalyzed the acetylation of H4K16, what enabled binding of bromodomain protein BRD4, subsequent recruitment of positive elongation factor PTEFb and the release of RNA Pol II from the promoter proximal pausing state into productive elongation [83]. A similar mechanism was shown to occur in *Drosophila* at the *hsp70* promoter upon heat shock [84]. In that case, JIL-1 mediated phosphorylation of H3S10 led to binding of 14-3-3 what led to recruitment of elongator protein 3 (Elp3), an H3K9 acetyltransferase important for the early transcriptional elongation step.

As discussed above, MSK1/2 are not the only kinases that mediate histone H3 phosphoacetylation in mammalian cells. It was show that human Mediator containing cdk8 subcomplex is stably associated with the acetyltransferase GCN5L. Upon serum induction CDK8 kinase phosphorylated H3S10 what stimulated the activity of GCN5L to acetylate H3K14 at the same histone tail at *p21*, *Egr1* and *c-Fos* promoters and coincided with their transcriptional activation. Knockdown of CDK8 resulted in the decrease in histone H3 phosphoacetylation levels at those promoters and abolished their expression [85]. However, it has not been yet determined whether 14-3-3 serves as a reader in the context of CDK8-mediated phosphoacetylation.

5. Models for phospho-histone H3 mediated regulation

The immediate neighborhood of serine 10 and serine 28 to lysine 9 and lysine 27, which often carry stable lysine methylation marks bound by factors associated with transcriptional repression, led to the hypothesis that histone phosphorylation may by a part of a 'phospho/methyl switch' mechanism, where phosphorylation of a give site would result in dissociation of a reader of the lysine methylation mark localized at the adjacent residue [86]. Indeed, it was demonstrated that during mitosis HP1 proteins α, β and γ are released from chromatin as a result of Aurora-B mediated phosphorylation of H3S10, probably to enable cohesin binding [87,88]. However, in another report H3S10 phosphorylation had only a moderate effect on HP1 binding to the H3K9me3 mark [89] and Mateescu et al. found that additional H3K14 acetylation is required for HP1 dissociation [90]. It is possible that *in vivo* other factors contribute to the release of HP1 from phosphomethylated histone H3 during mitosis. Notably, the reader protein 14-3-3 does not associate with H3-phosphorylated chromatin during mitosis [78,80]. One could speculate that a yet unidentified factor binds to the H3S10ph or the H3K9me3S10ph mark to contribute to initiation or maintenance of chromatin condensation. Alternatively, H3S10 phosphorylation at pericentromeric heterochromatin during mitosis might lead to transient transcriptional activation and formation of short RNAs. As potential substrates for the RNAi machinery these transcripts could be required to reinforce heterochromatic structures (reviewed in [91]).

In any case, H3K9me3 levels remain unchanged what suggest a potent mechanism for maintaining the epigenetic memory throughout cell division. This mechanism allows for the rapid reestablishment of the repressive state once mitosis is completed. Interestingly, an analogous 'H3T3phospho/H3K4methyl switch' seems to function to disable gene activation during mitotic division as it was demonstrated that haspin-mediated H3T3 phosphorylation leads to the dissociation of TFIID from H3K4me3-decorated promoters during mitosis [92].

Of note, the 'phospho/methyl switch' was shown to also occur upon signal induced phosphorylation of histone H3 during interphase. In particular, it was demonstrated that stress stimulation led to phosphorylation of H3S10 at the *HDAC1* promoter, resulting in dissociation of HP1γ from H3K9me2, followed by 14-3-3 recruitment and the onset of transcription [80]. Remarkably, the subsequent, transient gene activation took place in the presence of the repressive H3K9me2 mark and after the removal of the phosphate group from H3S10 the *HDAC1* promoter became repressed again (Fig. 3A). Similarly, H3Y41 phosphorylation by JAK2 was shown to release HP1α from chromatin, indicating that 'phospho/methyl switch' in response to extracellular signaling might be a more general mechanism to transiently override the repressive state [62].

As mentioned before, H3S28 is regulated in a similar way as H3S10 in terms of MAP kinase-mediated phosphorylation and located adjacent to K27, which can be tri-methylated and bound by polycomb repressive complexes PRC1 and PRC2. Polycomb group (PcG) proteins play an essential role in the maintenance of lineage specific gene expression patterns throughout cell division and are regulated by developmental signals [93]. Recently, it was demonstrated that phosphorylation of H3S28 upon stress and mitogenic stimulation as well as retinoic acid induced differentiation resulted in displacement of PcG proteins from a subset of their target promoters [94]. Dissociation of PRC led to transcriptional activation without removal of the H3K27me3 mark (Fig. 3B). This study provided a valuable insight into so far poorly understood regulation of PcG functions upon environmental signaling.

Interestingly, artificial targeting of MSK1 to the polycomb target gene *α-globin* reactivated its expression, what was due to displacement of PcG in response to phosphorylation of H3S28. Moreover, MSK1 induced acetylation of H3K27 at the *α-globin* promoter, suggesting that H3K27acS28ph dual mark antagonizes polycomb silencing [95]. This mechanism is particularly interesting in the context of previous studies demonstrating that the loss of PRC2 activity resulted in a global increase in acetylation of H3K27 [96]. However, until now it is not known how the H3S28ph mark is mechanistically linked to gene activation. It would be tempting to speculate that 14-3-3 proteins may serve as readers for this modification, as they bind H3S28ph peptides as strongly as H3S10phK14ac peptides in *in vitro* binding assays [81].

Of note, histone H3 phosphorylation was also demonstrated to control lysine methylation directly. Particularly, H3T6 phosphorylation by PKCβ upon androgen receptor signaling inhibits LSD1-mediated demethylation of H3K4, allowing for transcriptional activation [63]. In the same signaling pathway androgen activated protein-kinase-C-related kinase 1 (PRK1), phosphorylates H3T11 what in turn facilitates the removal of methylation marks from H3K9 by Jumonji domain-containing protein 2C (JMJD2C), again positively influencing transcription [64]. Finally, phosphorylation of H3S10 was shown to inhibit the enzymatic activity of the demethylase JMJD2A, which catalyzes the demethylation of H3K9me3 [97].

Taken together, histone H3 phosphorylation acts in concert with other histone modifications to modulate essential cellular processes *via* at least two different mechanisms. It can either constitute a binding platform for the regulators of transcriptional machinery or temporarily interfere with the binding or activity of factors targeted to adjacent residues.

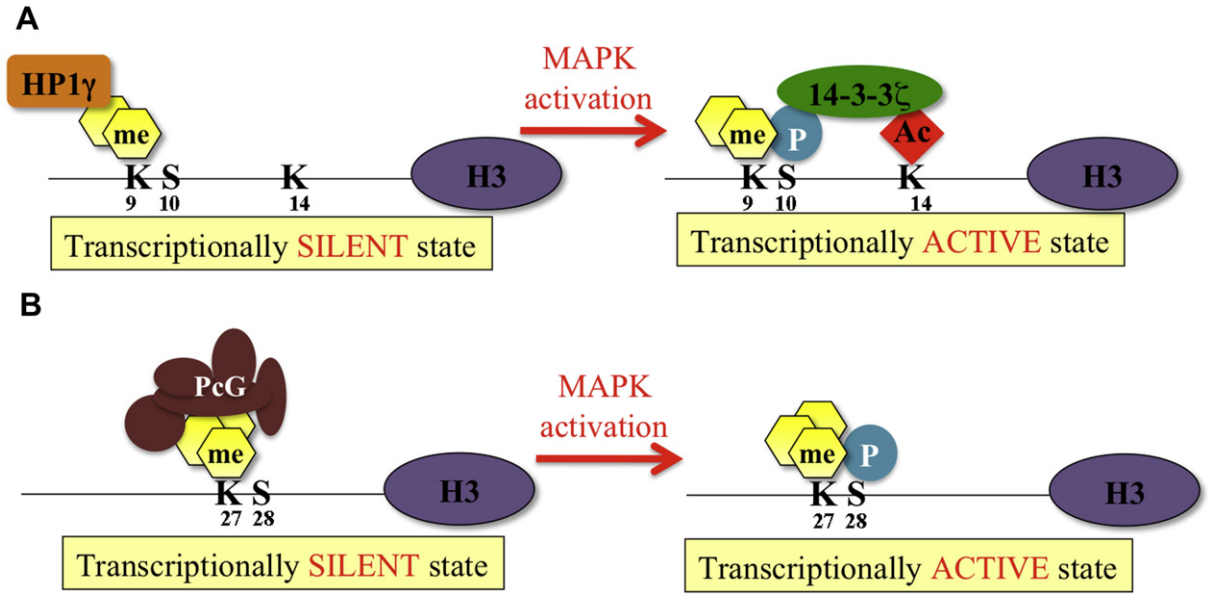

Fig. 3. Transient derepression by histone H3 phosphorylation. A. In the absence of signals the promoter is silent due to recruitment of HP1γ by local H3K9-dimethylation. Signal-dependent phosphorylation of H3S10 leads to dissociation of the HP1γ repressor, acetylation of H3K14 and recruitment of 14-3-3ζ. The resulting recruitment of RNA polymerase II and transcriptional activation occurs in the presence of the repressive H3K9me2 mark. Deacetylation, dephosphorylation and subsequent dissociation of 14-3-3ζ and binding of HP1γ reconstitute the repressed state of the promoter. B. In the silent state the promoter is decorated with H3K27m3 marks, which are bound by PcG. In response to extracellular signals H3S28 becomes phosphorylated and the PRCs dissociate. Transcriptional induction takes place in the presence of H3K27me3.

6. Conclusions

Histone H3 phosphorylation is a highly dynamic chromatin modification that impinges on the epigenetic landscape of eukaryotic cells in several ways. H3 phosphorylation contributes to the general condensation of chromatin during mitosis. In *Drosophila* phosphorylation of H3 counteracts heterochromatin spreading by antagonizing H3K9 methylation mediated by Su(var)3–9. In response to extracellular signals H3 phosphorylation triggers initiation or elongation of transcription and transiently activates genes by overriding repressive marks but without changing the epigenetic memory.

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