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Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation

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In recent years, the covalent modification of histone tails has emerged as a crucial step in controlling the transcription of eukaryotic genes. Phosphorylation of the serine 10 residue of the N-terminal tail of histone H3 is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition, this modification is important during interphase because it enables the transcription of an increasing number of genes that are activated as a consequence of a variety of cell-signaling events. The location of the serine 10 residue in close proximity to other modifiable amino acids in the histone H3 tail enables the possibility of an interaction between phosphorylation of serine 10 and methylation and/or acetylation of lysine 9 and lysine 14. Finally, the finding that the histone H3.3 variant, which has a conserved N-terminal tail, can replace histone H3 at sites of active transcription, adds a new layer of complexity and possibilities to the regulation of transcription through changes in chromatin structure.

The enormous length of the eukaryotic genome requires that it is packaged into a stable structure that can be replicated and propagated properly during mitosis and is sufficiently malleable and modifiable to enable access to genetic information. Such a structure is the composite material termed chromatin, which contains the entire genome and associated proteins [1]. Because DNA is compacted into a highly condensed and ordered structure, considerable interest has focused on how the transcriptional machinery gains access to the genes contained within chromatin and expresses them in an organized program, as is required in the processes of cellular differentiation and development. The alteration of chromatin organization, via covalent modification and/or remodeling of this structure, is thought to provide access to the genes for the transcription apparatus.

The location of the N-terminal tails of histone molecules outside of the relatively compact chromatin fiber makes them readily available for a variety of covalent post-translational modifications that are either thought to alter the local charged environment of the chromatin fiber, or act as a substrate for the binding of chromatin remodeling factors and/or transcription factors to regulate gene expression [2,3] (Figure 1).

The combinatorial pattern of N-terminal modifications results in a heterogeneous identity for each nucleosome

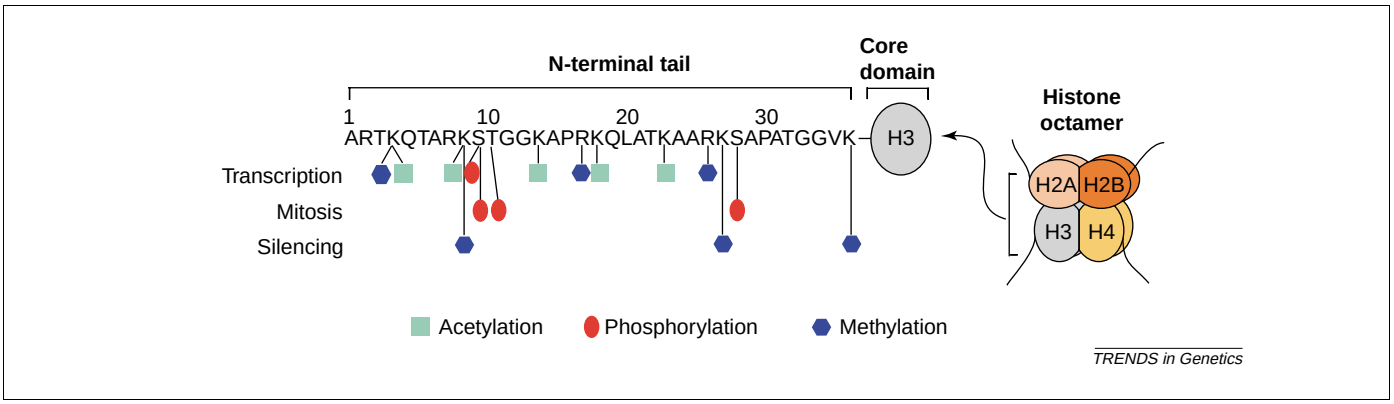

Figure 1. Modifications of the histone H3 N-terminal tail domain. These unordered domains protrude from the nucleosome and are modified by the cellular machinery via covalent modifications. The sites of the covalent modifications, acetylation, phosphorylation and methylation, are indicated. The associated cellular processes are indicated to the left of each set of modifications.

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that the cell interprets as a readable code from the genome to the cellular machinery for various processes to occur. This concept is commonly referred to as the 'histone code hypothesis' [3]. The first-studied and best-understood covalent histone modification was histone acetylation [4,5], and the list has grown to include phosphorylation [6], methylation [7], ubiquitination [8] and ADP-ribosylation [9]. Acetylation and methylation of different lysine (Lys) and arginine residues in histones H3 and H4 have been linked to either transcriptionally active or transcriptionally repressed states of gene expression [10], whereas phosphorylation of histone H3 was initially linked to chromosome condensation during mitosis [11,12] (Figure 1). More recently, evidence has accumulated that indicates the phosphorylation of histone H3 at serine 10 (Ser 10) has an important role in the transcriptional activation of eukaryotic genes in various organisms.

H3 phosphorylation during mitosis and meiosis

Ser 10 phosphorylation of histone H3 gained considerable interest when this modification was discovered to be associated with chromosome condensation and segregation during mitosis and meiosis [12]. Since this discovery, it has been found that mitosis-specific phosphorylation of histone H3 also occurs at Ser 28 [13] and at threonine 11 (Thr 11) [14]. It is unclear whether these modifications are causally linked. Histone H3 phosphorylation at Ser 10 begins during prophase, with peak levels detected during metaphase, ultimately followed by a general decrease in the amount of phosphorylation during the progression through the cell cycle to telophase [12]. A similar correlation can be observed during meiosis and a lack of Ser 10 histone H3 phosphorylation inhibits meiosis in *Tetrahymena thermophila* [11,15].

The development of antibodies specific for Ser 10-phosphorylated histone H3 isoforms facilitated the discovery that during mitosis histone phosphorylation originates in the pericentric heterochromatin and spreads throughout the genome during the G2–M phase transition [16]. Further analysis of histone H3 phosphorylation during mitosis revealed that phosphorylation is necessary for the initiation of chromosome condensation in mammalian [17] and *T. thermophila* [11] cells. Surprisingly, phosphorylation of Ser 10 in histone H3 is not required for cell-cycle progression in yeast, where phosphorylation of histone H2B might have a redundant role [18]. Studies in maize indicate that histone H3 Ser 10 phosphorylation during mitosis and meiosis begins late in prophase, after chromosome condensation has been initiated, and appears to be associated with chromosome cohesion rather than condensation [19].

Members of the aurora AIR2–Ipl1 kinase family have been found to govern histone H3 phosphorylation at Ser 10 during mitosis in several organisms [20–22]. The action of these particular kinases is thought to be required for the proper recruitment of the condensin complex and assembly of the mitotic spindle in a phosphorylated histone H3-dependent manner [22]. These kinases are counterbalanced by the activity of type 1 phosphatases (PP1) [18,23]. Regulation of the level of histone H3 phosphorylation via an interplay between the activities of protein kinase and phosphatase is thought to be the primary means of governing histone H3 phosphorylation during mitosis – promoting proper chromosomal condensation and segregation [24].

Histone H3 phosphorylation during transcription: a modification for rapid induction?

In 1991, Mahadevan and coworkers described the nucleosomal response [a rapid phosphorylation of histone H3 molecules concomitant with activation of the *c-fos* and *c-jun* immediate-early (IE) response genes] by stimulating fibroblast cells with growth factors, phorbol esters, inhibitors of protein synthesis and inhibitors of protein phosphatases (Figure 2). The observed timecourse of histone H3 phosphorylation mirrored the known expression profile of these genes, leading the authors to postulate a link between the phosphorylation of histone H3 and transcriptional activation [25]. Further studies demonstrated that this stimulation-dependent phosphorylation of histone H3 is a rapid and transient event and affects a population of phosphorylated histone H3 distinct from that normally detected in dividing cells. In addition, this fraction of IE response-phosphorylated histone H3 is susceptible to hyperacetylation [26], suggesting that these two histone modifications might be coupled during the activation of transcription via a MAP kinase-signaling cascade [27].

Further evidence for a role of Ser 10-histone H3 phosphorylation during the induction of transcription was obtained from several studies. Examination of patients with Coffin–Lowry syndrome (CLS), which is characterized by impaired transcriptional activation of *c-fos* and a loss of epidermal growth factor (EGF)-induced histone H3-phosphorylation, led to the suggestion that these patients are deficient in the ribosomal S6 serine–threonine kinase 2 (RSK2) [28], although recent studies support the conclusion that the mitogen- and stress-response kinases (MSK1 and MSK2) might be responsible for this effect [29,30] (Figure 2). Treatment of ovarian granulosa cells from immature rats with follicle-stimulating hormone (FSH) produces rapid histone H3 phosphorylation in a protein kinase A (PKA)-dependent manner, suggesting a role for histone phosphorylation during the establishment of a cellular differentiation pathway and a putative role in the transcription of genes involved in this pathway [31].

Analyzing the distribution of Ser 10-phospholylated histone H3 in the suprachiasmatic nucleus (SCN) of rats during stimulation with light, provided additional evidence for its role in gene expression. Light pulses induce prominent phosphorylated histone H3 staining in sections of the rat SCN, and this change in the distribution of Ser 10-phosphorylated histone H3 coincides with the change in the transcriptional profile of the IE gene *c-fos* and the circadian gene *Per1* [32]. Similar changes in Ser 10-histone H3 phosphorylation and gene expression have been observed after neuronal activation by agonists of dopamine (DA), muscarinic acetylcholine (mACh) and ionotropic glutamate (GLU) receptors in hippocampal neurons [33].

Interplay between phosphorylation and other histone H3 modifications

The Ser 10 residue in the N-terminal tail of histone H3 is located in a region of the protein that is also subject to

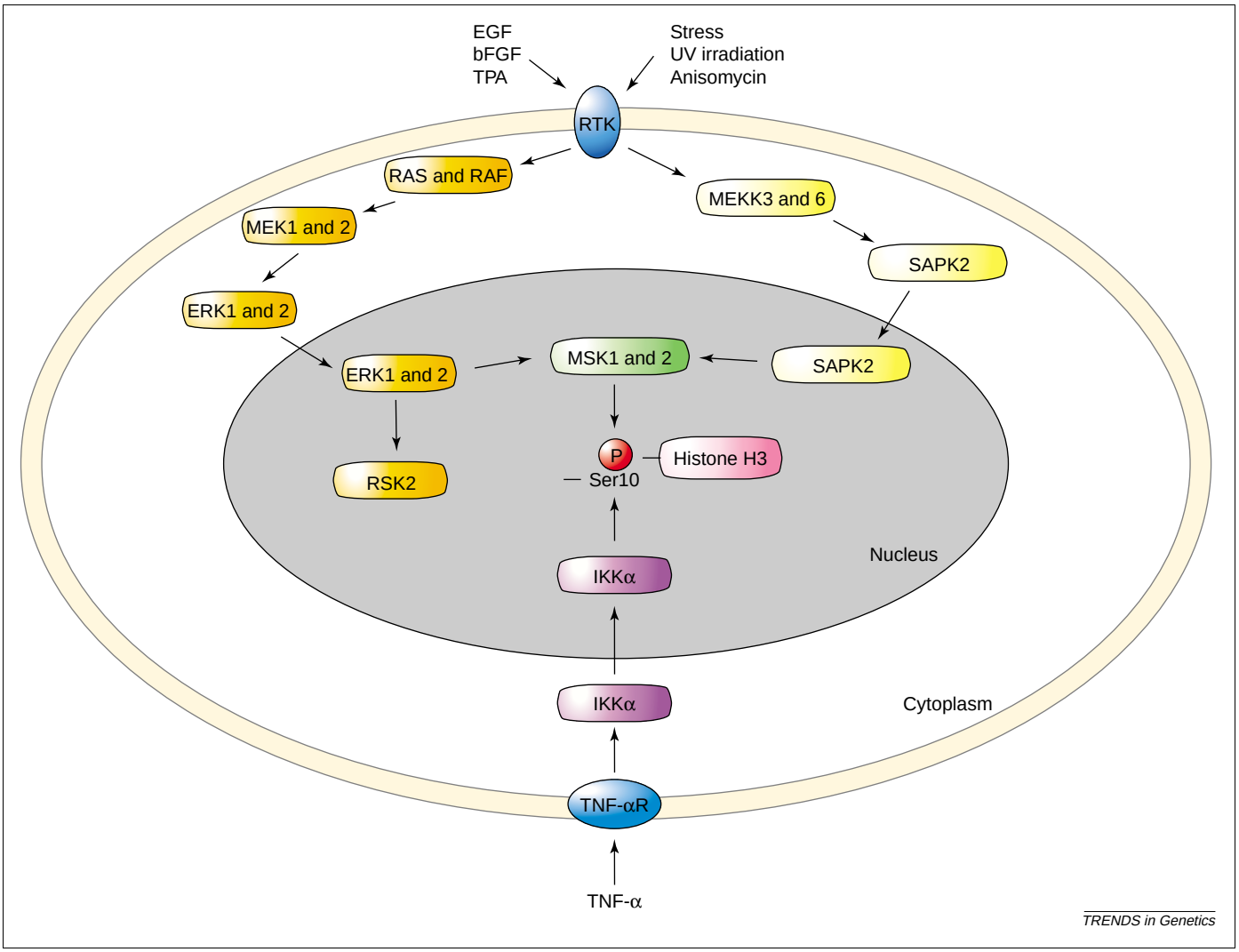

Figure 2. A summary of the cell-signaling cascades leading to histone H3 phosphorylation. The upper half of the diagram represents the main steps of two MAP-kinase cascades that are activated by mitogens, growth factors, stress or UV irradiation. In all of these examples, the extracellular signal eventually leads to activation of the MSK1 and MSK2 kinases, which phosphorylate histone H3 at Ser 10 and result in the activation of genes, such as *c-fos* and *c-jun*. The lower part of the diagram represents the main steps in cytokine-induced activation of NF-κB transcription; in this example, the IKKα kinase is responsible for phosphorylating histone H3 and switching on NF-κB expression. Abbreviations: EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; IKK, Iκ-kinase; MAPK, mitogen-activated protein–threonine kinase; MEK, mitogen-activated protein kinase–extracellular signal-regulated kinase kinase; MEKK, mitogen-activated protein kinase–ERK kinase kinase; MSK, mitogen- and stress-response kinase; NF-κB, nuclear factor-κB; RSK2, ribosomal S6 serine–threonine kinase; RTK, receptor tyrosine kinase; SAPK, stress-activated protein kinase; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

other covalent modifications (Figure 1). For example, Lys 9 and 14 can be acetylated as a prelude to transcriptional activation, whereas methylation of Lys 9 can lead to silencing and formation of heterochromatin. Therefore, these modifications might affect the ability of Ser 10 to be phosphorylated and vice versa, and evidence from *in vitro* and *in vivo* experiments supports this conclusion. For example, phosphorylation of a synthetic peptide by recombinant Ipl1–aurora kinase decreased when the Lys 9 residue was methylated, and phosphorylation of Ser 10 prevented methylation of Lys 9 by the SUV39H1 methyltransferase [a mammalian orthologue of the *Drosophila melanogaster* SU(VAR) 3-9]. Mouse embryonic fibroblasts with mutations in *suv39h1* have increased levels of Ser 10-phosphorylated histone H3, supporting the results obtained *in vitro* [7].

A relationship between acetylation and phosphorylation of histone H3 is also supported by a wealth of experimental data. Although these two modifications had been found to be associated with active transcription, initially, it was unknown whether they occurred on the same N-terminal tail of histone H3 or whether they were targeted to H3 molecules in different nucleosomes. Antibodies generated specifically to histone H3 tails that were both phosphorylated and acetylated provided the first direct proof that the di-modified H3 isoform existed *in vivo* [34,35]. *In vitro*, experiments revealed that the yeast GCN5 histone acetyltransferase displayed a preference for binding to a portion of the histone H3 tail that is pre-phosphorylated at the Ser 10 position [34–36]. Further resolution of the structure of the phosphohistone H3 N-terminal arm and GCN5 identified a single amino-acid side chain link between GCN5 and the phosphate group at the Ser 10 position of the H3 N-terminal peptide [36].

Nucleosome particles containing acetylated and phosphorylated histone H3 tails have been detected at the

promoters of several MAP-kinase-activated IE genes and at the promoters of *c-fos* pathway genes by chromatin immunoprecipitation (ChIP) assays in 10 T1/2 cells [34] – although only a subset of GCN5-regulated promoters require histone phosphorylation for transcriptional activation [36]. Taken together, these results suggest a synergistic mechanism of addition of each of these modifications to the N-terminal tails of histone H3 in the nucleosome particles at these promoters, whereby MAP-kinase signaling results in the phosphorylation of histone H3 at Ser 10. This phosphorylated N-terminal arm is then bound preferentially by GCN5, which acetylates the same histone H3 N-terminal tail at the Lys 14 position (Figure 3a). The acetylation of histone H3 leads to the induction of transcription. In yeast, the sucrose non-fermented 1 (Snf1) kinase, which regulates Ser 10 phosphorylation of histone H3 at the *INO1* promoter, was later discovered to act in concert with GCN5 to induce transcription in a phosphorylation first, acetylation second manner [37].

Acetylation versus phosphorylation: synergistic or parallel pathways?

Simultaneous studies on mouse fibroblast cells demonstrated that EGF stimulation leads to the phosphorylation and acetylation of the same histone H3 tail as a result of the nucleosomal response [35]. However, these modifications are each deposited via independent pathways, suggesting that, at least in some cases, histone H3 phosphorylation at Ser 10 is not just a signal for subsequent acetylation at Lys 14. Analysis of *c-jun* induction suggests that the presence of a phosphorylated histone H3 tail does not necessarily predispose that particular tail to acetylation at Lys 14. MAP-kinase-dependent histone H3 phosphorylation is detected at active, pre-acetylated loci on gene induction. In addition, the maintenance of acetylated histone H3 and H4 isoforms at a particular gene is a dynamic process that depends on an equilibrium shift between histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity; thus, establishing a mechanism whereby phosphorylation and acetylation at particular nucleosomes occur via parallel independent pathways. In this case, the presence of one modification does not necessarily predispose a histone tail to further modification (Figure 3b). This conclusion is supported by the observation that mutations in the genes encoding MSK1 and MSK2 kinases did not result in a detectable loss of histone H3 acetylation at IE genes, suggesting that the two events might be uncoupled [30].

Retinoic acid receptor β (*Rarβ*), an IE gene that is transcribed in response to stimulation of murine carcinoma cells with retinoic acid, also appears to be regulated in a manner similar to the *c-jun* IE gene in terms of its histone modifications [38]. Analysis of the mRARβ2 promoter reveals that this particular locus is constitutively H3-acetylated but not phosphorylated at Lys 9 and 14. H3 phosphorylation at the mRARβ2 promoter is directly induced by retinoic acid signaling, however, no corresponding change was observed in the H3 acetylation

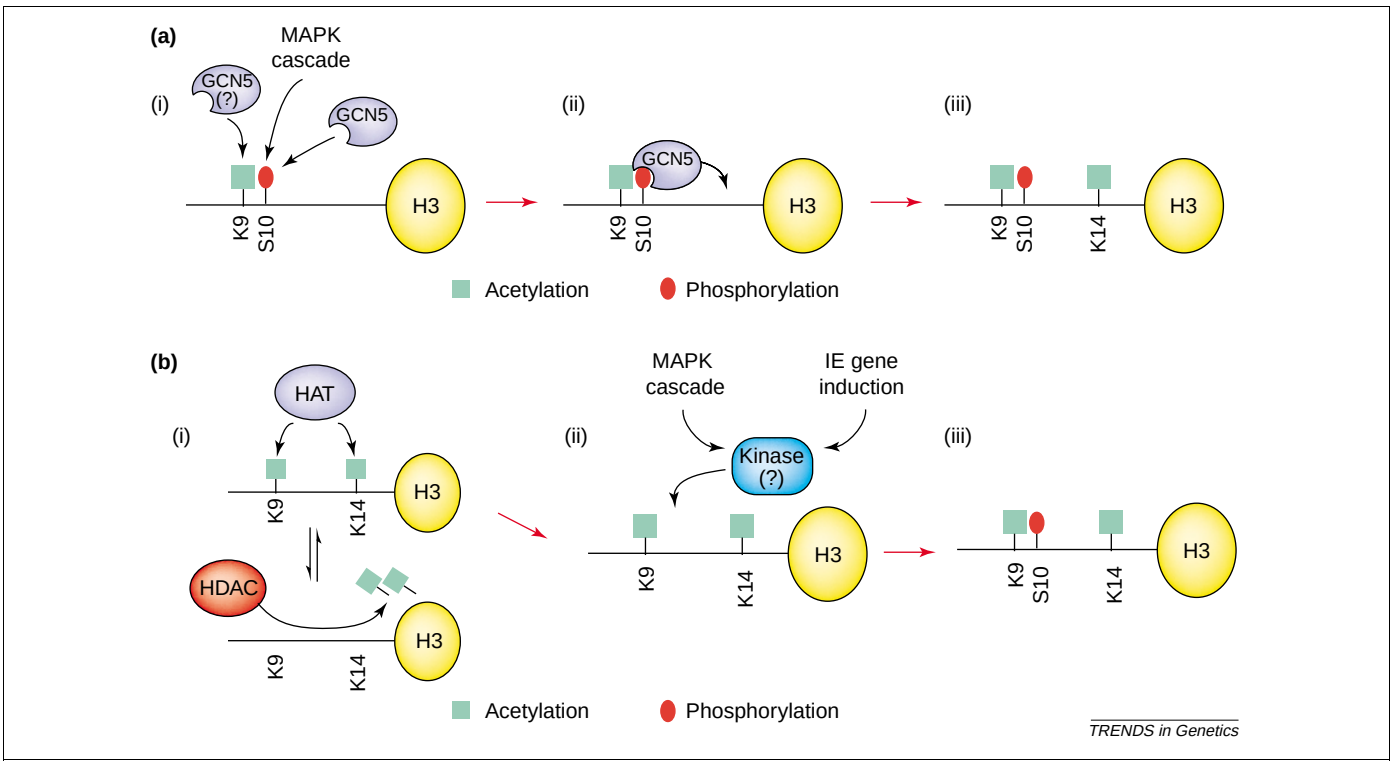

Figure 3. A simplified representation of the two pathways of modification of histone H3 N-terminal domains during gene activation. **(a)** The synergistic pathway of histone H3 phosphorylation, acetylation and subsequent gene induction. Histone H3–K9 is thought to be acetylated via GCN5 histone acetyltransferase (HAT) activity before phosphorylation at the Ser 10 position. (i) MAPK signaling results in the phosphorylation of histone H3 at Ser 10. (ii) The Ser 10-phosphorylated N-terminal tail increases binding affinity for GCN5. Once bound, the HAT activity of GCN5 results in acetylation of H3 at the K14 position. (iii) This results in transcription of the associated genes. **(b)** The parallel independent pathway of histone modification. (i) Acetylation of histone histone H3 at K9 and K14 is maintained by a competitive equilibrium between endogenous HAT and histone deacetylase (HDAC) activity. (ii) Kinase activity resulting from either MAPK-signaling cascades or immediate-early (IE) gene induction phosphorylates a pre-K9 and K14-acetylated histone H3 tail. (iii) Transcription of the associated genes with nucleosomes containing the di-acetylated, Ser 10-phosphoryated histone H3 N-terminal tail then proceeds. Abbreviations: K, lysine; MAPK, mitogen-activated protein–threonine kinase; Ser, serine.

profile. It appears that activation of the mRARβ2 promoter relies on histone phosphorylation rather than H3 acetylation for the induction of transcription, in agreement with the 'independent parallel' pathway of histone modification [39]. Concurrent analysis of other promoters in murine carcinoma-cell lines, such as the γ-actin promoter, indicates that they are not modified in the same way as mRARβ2 during retinoid stimulation, suggesting that there is a specific method of regulation of particular IE genes via histone modification [38].

The coupling of histone H3 phosphorylation at Ser 10 and acetylation at Lys 14 has also been observed during PKA-mediated transcriptional activation by FSH [40] and during cytokine-induced gene expression mediated by IκB kinase α (IKK-α) [41]; in this example, IKK-α regulates the cytokine-mediated activation of the NF-κB pathway (Figure 2) both by controlling the nuclear localization of NF-κB and by the direct phosphorylation of H3 [41,42]. However, it is unclear whether phosphorylation is a requirement for further modification via acetylation of Lys 14.

Histone H3 phosphorylation during transcriptional activation in *Drosophila*

Evidence from the analysis of gene expression in *Drosophila* has provided additional examples of genes that appear to follow the independent parallel pathway of histone modification during induction of transcription. Taking advantage of the heat-shock response of *Drosophila* (a well-defined means of global inactivation of transcription and induction of the heat-shock genes in response to thermal stress [43,44]) the genome-wide distribution of histone H3 Lys 14-acetylation and Ser 10-histone H3 phosphorylation was examined [45]. By analyzing polytene chromosomes that were prepared from heat shocked larvae, it was found that acetylated histone H3 and H4 at residues described as essential for transcription (i.e. Lys 14 of H3 and Lys 8 of H4) do not change their distribution. However, the distribution of Ser 10-phosphorylated histone H3 changes dramatically and is only detected at those loci that contain actively transcribing heat-shock genes. In addition, repression of transcription at non-heat-shock genes is accompanied by dephosphorylation of histone H3. These results suggest that the genomic distribution of histone H3 and H4 acetylation remains more or less static, whereas histone phosphorylation changes dynamically in a manner that is reminiscent of the transcriptional profile of the cell [45]. It also appears that the structure of the particular promoter itself might affect how histones are modified at a particular locus. Examination of the modification status of histone H3 at the promoters of GAL4-driven transgenes in *Drosophila* reveals that, whereas Ser 10-phosphorylated histone H3 molecules can be detected at active GAL4-regulated transgenes, active transgenes that do not bind TATA binding protein (TBP) are not H3-phosphorylated, raising the possibility that other histone modifications might have a role in their regulation [46]. One interesting result arising from this and previous studies [47] is the observation that antibodies against transcription-specific acetylated histones predominantly stained the non-transcribed 4′,6-diamidino-2-phenylindole (DAPI)-staining band regions of *Drosophila* polytene chromosomes. By contrast, histone phosphorylation is confined solely to the actively transcribed interband regions of polytene chromosomes. These results suggest that, in *Drosophila*, histone phosphorylation is intimately linked to transcriptional activation and that the presence of acetylated histones might not necessarily denote regions of actively transcribed genes [46].

Control of histone H3 phosphorylation by phosphatases

The sequence of events leading to histone H3 phosphorylation during induction of transcription could also possess another layer of complexity, where different kinases are recruited to specific promoters. Because specific transcription factors are known to bind to particular promoters, it could be inferred that these transcription factors might recruit or interact with unique kinases that serve to phosphorylate histone H3 at the nucleosomes of the promoter in question. In this article, we have described examples of different protein kinases that control the expression of specific genes after recruitment to their promoters. The presence of kinase–phosphatase pairs that maintain the proper state of histone H3 phosphorylation on nucleosomes during mitosis leads one to expect that a similar paradigm might be involved in the regulation of histone H3 phosphorylation during the processes of transcriptional activation and inactivation.

The kinase responsible for Ser 10 histone H3 phosphorylation in the *Drosophila* heat-shock genes has not been identified. A possible candidate is JIL-1, a novel tandem kinase with some similarity to members of the human MSK kinase family that has been shown to phosphorylate histone H3 at Ser 10 *in vivo* [48]. JIL-1 is known to associate with the male specific lethal (MSL) dosage-compensation complex in *Drosophila*, which is involved in the upregulation of X-chromosome transcription in male flies [49]. Hypomorphic mutations of JIL-1 have decreased levels of Ser 10-phosphorylated histone H3 and abnormal chromosomal morphology, suggesting a link between gene expression and maintenance of chromatin architecture [50]. Whether JIL-1 is involved in transcriptional activation by phosphorylation of histone H3 at specific *Drosophila* promoters is unknown; however, dephosphorylation seems to have a crucial role in this process. At the normal cell temperature, all sites on polytene chromosomes that correspond to actively transcribed genes contain Ser 10 phosphorylated histone H3. After heat shock, transcriptional activation of the heat-shock genes is accompanied by *de novo* histone H3 phosphorylation, whereas this modification disappears from the rest of the genome. Treatment of cells with phosphatase type 2A (PP2A) inhibitors, or mutations in PP2A-encoding genes, interfere with this genome-wide dephosphorylation of histone H3. The sites of histone H3 phosphorylation that do not contain heat-shock genes remain transcriptionally active during heat shock in PP2A mutants, suggesting that activation and repression of gene expression during heat shock depend on the phosphorylation status of histone H3, which might be regulated by changes in PP2A activity [51].

Conclusions

Despite numerous studies examining the possible correlation between the phosphorylation of histone H3 and a transcriptionally active state, it is still not clear whether this modification has a direct functional role in transcription or whether it is only a prerequisite for further modification via acetylation of Lys 14.

Current evidence suggests that, at least in some cases, Ser 10 phosphorylation is necessary and sufficient for transcriptional activation without acetylation of additional residues. The mechanism by which this modification affects the chromatin structure to enable transcriptional activation is not understood. A distinct possibility is that histone H3 phosphorylation alters the local environment, increasing the accessibility of the chromatin fiber to facilitate access of the transcription machinery. This seems to contradict the observation that histone H3 phosphorylation at Ser 10 is essential for chromosomal condensation during mitosis. One possible explanation is that the mitotic signal requires subsequent phosphorylation of Ser 28 and Thr 11, and that these two modifications enable the distinction between condensation and transcription to be made. An alternative possibility is that Ser 10 phosphorylation might serve as a signal for the recruitment of specific proteins, such as chromosome condensation-promoting factors to the chromosome arms during mitosis or transcription-promoting factors to an associated gene for transcription. This recruitment of transcription-promoting factors is reminiscent of the role of histone H3 phosphorylation in the synergistic mechanism of histone H3 modification. In support of this, recent work [52] has demonstrated that histone acetylation and phosphorylation act as directed binding sites that are recognized by the transcriptional machinery, rather than acting as modifications that alter the local charge of the chromatin environment.

A second issue that needs to be explored further is the role of histone H3 variants during transcriptional activation. Recent results [53,54] suggest that transcription of rDNA arrays in *Drosophila* correlates with the replacement of histone H3 for the H3.3 variant. Perhaps the *de novo* phosphorylation that is observed at sites of active transcription might affect histone H3.3 rather than histone H3, whereas the Ser 10 phosphorylation leading to chromosome condensation is specific for histone H3. This could account for the multiple observations of histone H3 acetylation rather than phosphorylation as a mechanism for transcriptional activation of yeast genes [34,36,37]. Because the histone H3.3 variant is the only form of histone H3 that is found in yeast [53,54] an exchange between histone H3 and H3.3 (and therefore histone H3 phosphorylation) might not be required for the activation of transcription in this organism.

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References

1 Wolffe, A. (1998) *Chromatin: Structure and Function*, Academic Press
2 Turner, B.M. (1998) Histone acetylation as an epigenetic determinant of long-term transcriptional competence. *Cell. Mol. Life Sci.* 54, 21–31
3 Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* 403, 41–45
4 Brownell, J.E. *et al.* (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* 84, 843–851
5 Allfrey, V.G. *et al.* (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 51, 786–794
6 Paulson, J.R. and Taylor, S.S. (1982) Phosphorylation of histones 1 and 3 and nonhistone high mobility group 14 by an endogenous kinase in HeLa metaphase chromosomes. *J. Biol. Chem.* 257, 6064–6072
7 Rea, S. *et al.* (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593–599
8 Levinger, L. and Varshavsky, A. (1982) Selective arrangement of ubiquitinated and D1 protein-containing nucleosomes within the *Drosophila* genome. *Cell* 28, 375–385
9 Jump, D.B. *et al.* (1979) Nuclear protein modification and chromatin substructure. 3. Relationship between poly (adenosine diphosphate) ribosylation and different functional forms of chromatin. *Biochemistry* 18, 983–990
10 Fischle, W. *et al.* (2003) Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* 15, 172–183
11 Wei, Y. *et al.* (1998) Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in Tetrahymena. *Proc. Natl. Acad. Sci. U. S. A.* 95, 7480–7484
12 Gurley, L.R. *et al.* (1978) Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. *Eur. J. Biochem.* 84, 1–15
13 Goto, H. *et al.* (1999) Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. *J. Biol. Chem.* 274, 25543–25549
14 Preuss, U. *et al.* (2003) Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase. *Nucleic Acids Res.* 31, 878–885
15 Wei, Y. *et al.* (1999) Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* 97, 99–109
16 Hendzel, M.J. *et al.* (1997) Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* 106, 348–360
17 Van Hooser, A. *et al.* (1998) Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation. *J. Cell Sci.* 111, 3497–3506
18 Hsu, J.Y. *et al.* (2000) Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102, 279–291
19 Kaszas, E. and Cande, W.Z. (2000) Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin. *J. Cell Sci.* 113, 3217–3226
20 de la Barre, A.E. *et al.* (2000) Core histone N-termini play an essential role in mitotic chromosome condensation. *EMBO J.* 19, 379–391
21 De Souza, C.P. *et al.* (2000) Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*. *Cell* 102, 293–302
22 Giet, R. and Glover, D.M. (2001) Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* 152, 669–682
23 Murnion, M.E. *et al.* (2001) Chromatin-associated protein phosphatase 1 regulates aurora-B and histone H3 phosphorylation. *J. Biol. Chem.* 276, 26656–26665
24 Hans, F. and Dimitrov, S. (2001) Histone H3 phosphorylation and cell division. *Oncogene* 20, 3021–3027
25 Mahadevan, L.C. *et al.* (1991) Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* 65, 775–783
26 Barratt, M.J. *et al.* (1994) Mitogen-stimulated phosphorylation of histone H3 is targeted to a small hyperacetylation-sensitive fraction. *Proc. Natl. Acad. Sci. U. S. A.* 91, 4781–4785
27 Thomson, S. *et al.* (1999) MAP kinase-mediated signalling to

nucleosomes and immediate-early gene induction. *Semin. Cell Dev. Biol.* 10, 205–214
28 Sassone-Corsi, P. *et al.* (1999) Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. *Science* 285, 886–891
29 Strelkov, I.S. and Davie, J.R. (2002) Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts. *Cancer Res.* 62, 75–78
30 Soloaga, A. *et al.* (2003) MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J.* 22, 2788–2797
31 DeManno, D.A. *et al.* (1999) Follicle-stimulating hormone promotes histone H3 phosphorylation on serine-10. *Mol. Endocrinol.* 13, 91–105
32 Crosio, C. *et al.* (2000) Light induces chromatin modification in cells of the mammalian circadian clock. *Nat. Neurosci.* 3, 1241–1247
33 Crosio, C. *et al.* (2003) Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. *J. Cell Sci.* 116, 4905–4914
34 Cheung, P. *et al.* (2000) Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol. Cell* 5, 905–915
35 Clayton, A.L. *et al.* (2000) Phosphoacetylation of histone H3 on *c-fos*- and *c-jun*- associated nucleosomes upon gene activation. *EMBO J.* 19, 3714–3726
36 Lo, W.S. *et al.* (2000) Phosphorylation of serine 10 in histone H3 is functionally linked *in vitro* and *in vivo* to Gcn5-mediated acetylation at lysine 14. *Mol. Cell* 5, 917–926
37 Lo, W.S. *et al.* (2001) Snf1 – a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. *Science* 293, 1142–1146
38 Lefebvre, B. *et al.* (2002) Phosphorylation of histone H3 is functionally linked to retinoic acid receptor beta promoter activation. *EMBO Rep.* 3, 335–340
39 Thomson, S. *et al.* (2001) Independent dynamic regulation of histone phosphorylation and acetylation during immediate-early gene induction. *Mol. Cell* 8, 1231–1241
40 Salvador, L.M. *et al.* (2001) Follicle-stimulating hormone stimulates protein kinase A-mediated histone H3 phosphorylation and acetylation leading to select gene activation in ovarian granulosa cells. *J. Biol. Chem.* 276, 40146–40155
41 Yamamoto, Y. *et al.* (2003) Histone H3 phosphorylation by IKK-α is critical for cytokine-induced gene expression. *Nature* 423, 655–659
42 Anest, V. *et al.* (2003) A nucleosomal function for IκB kinase-α in NF-κB-dependent gene expression. *Nature* 423, 659–663
43 Mirault, M.E. *et al.* (1978) The effect of heat shock on gene expression in *Drosophila melanogaster*. *Cold Spring Harb. Symp. Quant. Biol.* 42, 819–827
44 Spradling, A. *et al.* (1975) Analysis of drosophila mRNA by *in situ* hybridization: sequences transcribed in normal and heat shocked cultured cells. *Cell* 4, 395–404
45 Nowak, S.J. and Corces, V.G. (2000) Phosphorylation of histone H3 correlates with transcriptionally active loci. *Genes Dev.* 14, 3003–3013
46 Labrador, M. (2003) Phosphorylation of histone H3 during transcriptional activation depends on promoter structure. *Genes Dev.* 17, 43–48
47 Pile, L.A. and Cartwright, I.L. (2000) GAGA factor-dependent transcription and establishment of DNase hypersensitivity are independent and unrelated events *in vivo*. *J. Biol. Chem.* 275, 1398–1404
48 Jin, Y. *et al.* (1999) JIL-1: a novel chromosomal tandem kinase implicated in transcriptional regulation in *Drosophila*. *Mol. Cell* 4, 129–135
49 Jin, Y. *et al.* (2000) JIL-1, a chromosomal kinase implicated in regulation of chromatin structure, associates with the male specific lethal (MSL) dosage compensation complex. *J. Cell Biol.* 149, 1005–1010
50 Wang, Y. *et al.* (2001) The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. *Cell* 105, 433–443
51 Nowak, S.J. and Corces, V.G. (2003) Protein phosphatase 2A activity affects histone H3 phosphorylation and transcription in *Drosophila melanogaster*. *Mol. Cell. Biol.* 23, 6129–6138
52 Agalioti, T. *et al.* (2002) Deciphering the transcriptional histone acetylation code for a human gene. *Cell* 111, 381–392
53 Ahmad, K. and Henikoff, S. (2002) The histone variant h3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* 9, 1191–1200
54 Ahmad, K. and Henikoff, S. (2002) Histone H3 variants specify modes of chromatin assembly. *Proc. Natl. Acad. Sci. U. S. A.* 99 (Suppl. 4), 16477–16484