Minireview

MAP kinase-mediated phosphoacetylation of histone H3 and inducible gene regulation

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Abstract That signalling pathways, particularly the mitogen-activated protein kinase cascades, elicit modification of chromatin proteins such as histone H3 by phosphorylation and/or acetylation concomitant with gene activation is now well established. The picture that is emerging is one of a complex and dynamic pattern of multiple modifications at the H3 tail. Here, we review the inducible gene systems where H3 modifications have been reported and re-evaluate the controversy as to the kinase(s) that phosphorylates it as well as the proposed coupling between H3 phosphorylation and acetylation.

Key words: Acetylation; Immediate-early gene induction; Mitogen-activated protein kinases; Mitogen- and stress-activated protein kinase; Phosphorylation; Ribosomal S6 kinase

1. Introduction

When cells receive external signals initiated by growth factors, cytokines, pharmacological agents or stresses, intracellular signalling pathways are activated which rapidly alter patterns of gene expression. The first phase of this is the induction of immediate-early (IE) genes, those that are directly connected to intracellular signalling systems and do not require new protein synthesis. Much emphasis and research effort in the signal transduction field has focused on identifying the physiologically relevant targets of such pathways, particularly the transcription factors and co-activators at the enhancers and promoters of individual genes. More recently, it has become clear that chromatin structure plays an important role in eukaryotic gene regulation. An increasing body of evidence indicates that as well as directing the phosphorylation of upstream transcription factors and co-activators controlling IE genes, signalling pathways, particularly the mitogen-activated protein (MAP) kinase cascades, also act directly on chromatin proteins such as histone H3 and high mobility group HMG-14 (HMGN1 revised nomenclature [1]) to modify chromatin concomitant with gene induction.

Alterations in chromatin structure can be achieved by two main mechanisms: the post-translational modification of histones in their amino-terminal tails by acetylation, phosphorylation, methylation and ubiquitination (see [2] for a review and references therein), or remodelling of nucleosomes via the ATP-dependent chromatin remodelling complexes such as Swi/Snf (see [3–5] for reviews). Chromatin remodelling complexes are not within the scope of this review. Post-translational histone modifications may affect chromatin structure by altering histone–DNA contacts. The modifications (or combinations of modifications) may also act as binding platforms/recognition motifs for interaction with other proteins that may provide further enzyme activity or recruit additional regulatory proteins, as proposed by the histone code hypothesis [6–8]. So far, two types of specific protein domains, the bromodomain that binds to acetyl-lysine (reviewed in [9,10]) and the chromodomain that binds to methyl-lysine (reviewed in [11]) have been identified.

Acetylation has been the most extensively studied modification and the first indications that inducible genes may exhibit enhanced acetylation upon activation came from the work of Allfrey and colleagues looking at the c-*fos* and c-*myc* genes [12,13]. More recently, phosphorylation of histone H3 concomitant with IE gene induction has been demonstrated [14], and the MAP kinase cascades that deliver this response have been elucidated [15–17]. These studies eventually led to the remarkable demonstration that both modifications were targeted to the same histone H3 tails and that these were directly located on IE gene chromatin [18–20]. Here, we review the different inducible gene systems where histone H3 phosphorylation and acetylation have been reported, re-evaluate the controversy as to the kinase that phosphorylates it and finally, discuss the proposed coupling between the two modifications.

2. Nucleosome modifications induced by extracellular stimuli

Extracellular stimuli are connected to IE genes by several possible

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Abbreviations: BMK, big mitogen-activated protein kinase; CBP, CREB binding protein; CREB, cAMP-responsive element binding protein; EGF, epidermal growth factor; ERK/MAPK, extracellular signal-regulated kinase/mitogen-activated protein kinase; FSH, follicle-stimulating hormone; HAT, histone acetyltransferases; HDAC, histone deacetylase; HMG, high mobility group; IE gene, immediate-early gene; JNK/SAPK, c-Jun amino-terminal kinase/stress-activated protein kinase; MAP kinase, mitogen–activated protein kinase; MAPKAP-K1/RSK, MAP kinase-activated protein kinase-1/ribosomal S6 kinase; MSK, mitogen- and stress-activated protein kinase; PKA, protein kinase A

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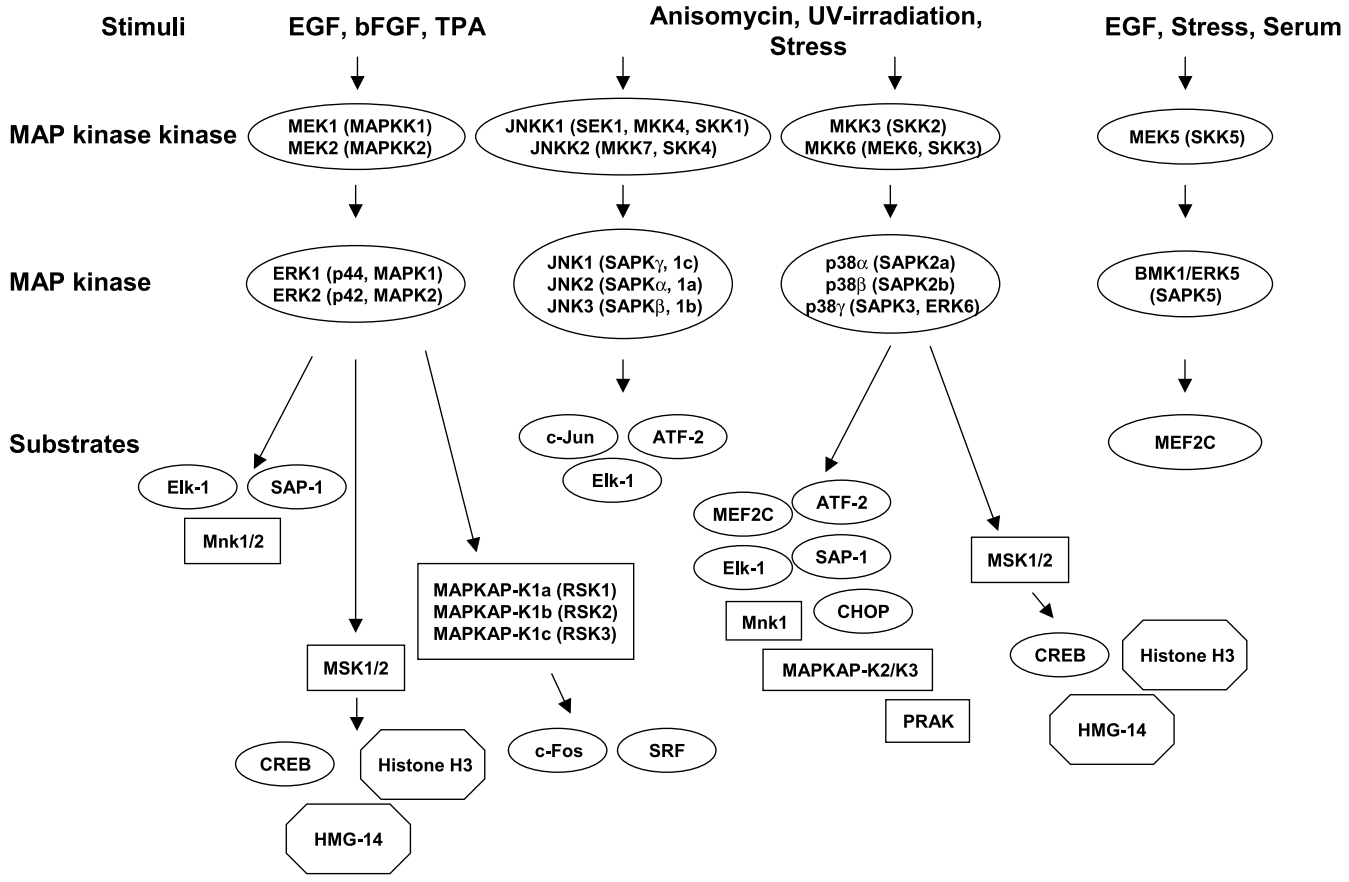

Fig. 1. Schematic representation of the four characterised mammalian MAP kinase cascades and target substrates including downstream kinases, transcription factors and nucleosomal proteins, modified from [27]. The four cascades are the ERK/MAPK pathway, the JNK/SAPK pathway, the p38/reactivating kinase (RK) pathway, and the BMK/ERK5 pathway. Kinase substrates downstream of the MAP kinases themselves are shown as rectangles. Transcription factor substrates are shown as ovals and nucleosomal proteins are shown as octagons. For further details see [27] and references therein.

signalling pathways. These include the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway (reviewed in [21]), the nuclear factor κB (NF-κB) pathway (reviewed in [22]) and the MAP kinase signalling pathways (reviewed in [23,24]). We will concentrate mainly on MAP kinase-mediated signalling to chromatin since this is one of the most extensively studied systems to date, in particular with respect to histone H3 modifications.

The main experimental models for inducible phosphorylation and acetylation of histone H3 linked to activation of inducible genes have been the c-*jun* and c-*fos* proto-oncogene families [18–20,25,26]. These genes are activated rapidly and transiently, without a requirement for new protein synthesis, and are therefore linked directly to signalling cascades. Present indications are that these genes are under tight quantitative control, induction being highly characteristic and reproducible in terms of the extent and duration of expression in response to different stimuli (reviewed in [27]).

3. MAP kinase pathways

There are currently four MAP kinase cascades that are differentially activated depending on stimulus: (1) the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) 1/2 pathway, (2) the c-Jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway, (3) the p38 pathway and (4) the big mitogen-activated protein kinase BMK/ERK 5 pathway (shown schematically in Fig. 1). The signalling network leading to activation of the MAP kinases themselves and some of their downstream targets has been extensively reviewed elsewhere (see [27] and references therein).

Activation of the MAP kinase pathways leads ultimately to the phosphorylation of transcription factors bound to their regulatory elements in the promoters of target genes.

Phosphorylation of these transcription factors is crucial for gene activation [28], but it is now clear that upstream elements and transcription factors can also direct acetylation to nucleosomes of IE gene chromatin. Co-activators such as p300/CBP are histone acetyltransferases (HATs) [29,30] that bind to a variety of transcription factors such as c-Jun and c-Fos (see [31] and references therein). CBP itself can then recruit further HAT activity by association with p/CAF [32]. The MAP kinase-dependent recruitment of co-activators with HAT activity to sequence-specific regulatory elements provides a possible mechanism for the targeted localised alterations in histone acetylation levels that have been observed in a number of different systems [33–40].

4. Histone H3 phosphorylation

Serines 10 and 28 on the histone H3 tail are both preceded by the same three amino acids (alanine-arginine-lysine) and both these phosphorylatable motifs are very highly conserved through evolution, being identical in yeast and man. There are two distinct modes of histone H3 phosphorylation, mitotic

and stimulus-inducible phosphorylation, both of which can utilise these two serine residues. Currently identified stimulus-induced and mitotic H3 kinases are summarised in Table 1.

4.1. Mitotic H3 phosphorylation

It has long been observed that serine 10 on histone H3 is very highly phosphorylated on condensed chromosomes during mitosis ([41–44], reviewed in [45]). More recently serine 28 has been identified as a second site of phosphorylation in mammals that also occurs during chromosome condensation at early mitosis [46]. Mitotic phosphorylation of histone H3 on serine 10 is mediated by members of the Aurora kinase family such as the Ipl1 kinase in yeast and nematodes [47], Aurora B in *Drosophila* [48] and Aurora B (and A) kinases in mammals ([49] and references therein). Aurora B is also implicated in mitotic serine 28 phosphorylation [50]. In in vitro assays a histone H3 serine 28 kinase activity has also been detected in yeast whole cell extracts [51]. Mitotic H3 phosphorylation is not discussed further here, but the major differences between mitotic and stimulus-induced modes are that the former is extensively observed throughout condensed chromosomes, whereas the latter is transient, affects a minute fraction of nucleosomes and is associated with active genes (discussed further below).

4.2. Stimulus-induced histone H3 phosphorylation

The first link between intracellular signalling pathways and phosphorylation of chromatin-associated proteins came from ^{32}P labelling studies in mouse fibroblasts. Diverse stimuli, including growth factors and protein synthesis inhibitors, all of which activate MAP kinase cascades, elicit the rapid and transient phosphorylation of H3 on serine 10 [52] and also HMG-14 on serine 6 [53], collectively called the nucleosomal response. The extent and duration of the nucleosomal response correlates with IE gene induction suggesting a role for these modifications in transcriptional activation [31]. The fraction of H3 that is phosphorylated at the G_0/G_1 transition is minute and is also highly susceptible to hyperacetylation induced by histone deacetylase (HDAC) inhibitors such as butyrate [14] and trichostatin A [18]. ^{32}P labelling studies of butyrate-treated cells [14] provided the first indication that multiple modifications (phosphorylation and acetylation) are targeted to the same histone H3 tail and may be important for IE gene induction.

A number of systems where inducible H3 phosphorylation (Ser10) and acetylation is associated with activated inducible genes have now been reported and are summarised in Table 2. Furthermore, MAP kinase-mediated phosphorylation of serine 28, the second site of phosphorylation in the H3 tail, has also been demonstrated upon stimulation with UV-B [54,55],12-*O*-tetradecanoylphorbol 13-acetate (TPA) and anisomycin ([56]; M.H. Dyson and L.C. Mahadevan, unpublished data) and epidermal growth factor (EGF) (M.H. Dyson and L.C. Mahadevan, unpublished data). We are presently investigating whether serine 28 phosphorylation is also targeted to IE gene nucleosomes concomitant with gene activation (M.H. Dyson and L.C. Mahadevan, unpublished data). No inducible serine 28 phosphorylation has yet been reported in yeast.

5. MAP kinase-activated histone H3 kinases

The use of relatively specific MAP kinase and other inhibitors [57–59] has allowed dissection of the signalling pathways/mechanisms involved in histone H3 (and HMG-14) phosphorylation in response to diverse stimuli. The ERK pathway is specifically inhibited by the flavone compound PD098059 and U0126 compound which inhibit the activation of MEK1/2, the upstream activator of ERKs [60,61]. The p38 pathway is inhibited by SB203580, which specifically binds to and inhibits p38 MAP kinase itself [62]. Studies with such inhibitors have shown that in mammalian cells H3 (and HMG-14) phosphor-

Table 1
Identified stimulus-induced and mitotic histone H3 kinases

Species/cells	Stimulus	Pathway	Kinase	H3 site	Reference
Drosophila melanogaster	X dosage compensation	–	JIL-1	Ser10	[75]
Human Coffin–Lowry fibroblasts	EGF	ERK	RSK2	Ser10	[70]
Drosophila melanogaster	–	–	JIL-1	Ser10	[76]
Mouse C3H 10T1/2 fibroblasts	Arsenite	p38	MSK1	Ser10	[87]
Mouse C3H 10T1/2 fibroblasts	sAn	p38	MSK1	Ser10	[17]
Mouse JB6 cells	UVB	p38 ERK JNK	MSK1	Ser28	[54]
Rat ovarian granulosa cells	FSH	PKA	PKA	Ser10	[77]
NIH 3T3 cells/COS-1 cells	EGF	ERK	RSK2	Ser10	[66]
Saccharomyces cerevisiae	Low glucose/sucrose present	sip/gal proteins?	Snf1	Ser10	[51]
Human promyelocytic leukaemia	Arsenite	MAPK?	MSK1/RSK2?	Ser10	[88]
Aspergillus nidulans	Mitotic	NIMXcdc2	NIMA	Ser10	[89]
Caenorhabditis elegans	Mitotic	–	AIR-2	Ser10	[47]
Saccharomyces cerevisiae	Mitotic	–	Ipl1p	Ser10	[47]
Xenopus oocyte	Mitotic	INCENP?	pEg2 Aurora A	Ser10	[90]
Drosophila melanogaster	Mitotic	INCENP	Aurora B	Ser10	[91]
Human HeLa cells	Mitotic	cdc2 blocks phosphatase	Aurora B	Ser28	[50]
Xenopus oocyte	Mitotic	Loss of pp1 activity?	Aurora B	Ser10	[92]
Mouse 3T3	Mitotic	–	Aurora B/A	Ser10	[49]
Drosophila melanogaster	Mitotic	–	Aurora B	Ser10	[48]
Human cells	Mitotic	–	Nek6	Ser10	[93]
Human MCF-7 breast cancer	Mitotic	–	PAK1	Ser10	[94]

Abbreviations: AIR-2, Aurora/Ipl1-related serine/threonine protein kinase 2; ERK, extracellular signal-regulated kinase; FSH, follicle-stimulating hormone; INCENP, inner centromere protein; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase 1; MSK1, mitogen- and stress-activated protein kinase 1; NEK6, NIMA-related expressed kinase 6; NIMA, Never In Mitosis A; PAK1, p21 activated kinase 1; PKA, protein kinase A; pp1, protein phosphatase 1; RSK2, ribosomal S6 kinase 2; sAn, sub-inhibitory anisomycin.

ylation (as well as IE gene induction) is mediated via the ERK or p38 MAP kinase pathways depending on the stimulus used, but not the JNK/SAPK pathway [15–17]. Although these two pathways deliver signals to histone H3 and HMG-14, phosphorylation is effected by downstream kinases and not by MAP kinases themselves (see the signalling cascade diagram, Fig. 1). The subject of much recent contention, two downstream kinases, MAP kinase-activated protein kinase-1/ribosomal S6-kinase (MAPKAP-K1b/RSK2) and mitogen- and stress-activated protein kinase (MSK1/2), have been suggested as the mammalian histone H3/HMG-14 kinases. As discussed below, recent studies are casting doubt on the physiological role of RSK2 as the ligand-stimulated H3 kinase. Note that although the histone H3 phosphorylation sites and MAP kinase cascades are conserved in yeast, the downstream equivalents of these inducible histone H3 kinases are missing, suggesting that this mode of chromatin modification arose more recently in evolution.

6. MAPKAP-K1b/RSK2

The MAPKAP-K1/RSKs, of which there are currently four identified isoforms in humans, MAPKAP-K1a, b and c, or RSK1, 2 and 3 (reviewed in [63]) and RSK4 [64], were the first downstream effector kinases identified. They are activated by the ERK pathway and this activation is inhibited by PD098059 ([65], reviewed in [31]). There are some reports that UV radiation can lead to phosphorylation and activation of RSK2 in some cell types [66,67], though the mechanism remains contentious.

Mutations in one of these kinases, RSK2/MAPKAP-K1b [68], is found in the human genetic disease Coffin–Lowry syndrome (CLS). Much of the evidence purporting to show that RSK2 is a ligand-stimulated H3 kinase comes from studies using fibroblasts established from CLS patients. In these cells only the RSK2 isoform is inactive and EGF-stimulated CREB phosphorylation and c-*fos* induction were reported to be defective, although these responses to UV radiation were reportedly normal [69]. By contrast, stimulation of CLS fibroblasts with EGF, serum or UV radiation reportedly failed to produce a H3 phosphorylation response [70]. Similar results for EGF-stimulated histone H3 phosphorylation were observed in mouse embryonic stem cells in which the RSK2 gene was knocked out by homologous recombination; moreover, trans-

Table 2
Genes associated with stimulus-induced phosphorylation and/or acetylation

Species/cells	Stimulus	Genes	Modification	Reference
Mouse NIH3T3 cells/NIH3T3 SRE-FosHA/SRE-lacZ reporter cell lines	TPA, sAn serum	SRE-fosHA, SRE-lacZ	AcH4	[35]
Mouse C3H 10T1/2 fibroblasts	TPA	c-*fos*, c-*myc*	PhosH3 Ser10[a]	[25]
Mouse C3H 10T1/2 fibroblasts	EGF	c-*fos*	Phos/AcH3 Ser10/Lys14	[19]
Mouse C3H 10T1/2 fibroblasts	EAn, EGF	c-*jun*, c-*fos*	Phos/AcH3 Lys9/Ser10	[18]
Mouse C3H 10T1/2 fibroblasts	sAn	c-*jun*	Phos/AcH3 Lys9/Ser10, AcH3 Lys9 and/or Lys14[b], AcH4	[20]
Drosophila salivary glands	Heat shock	Heat shock loci	PhosH3 Ser10[a]	[95]
Rat ovarian granulosa cells	FSH	*SGK*, *inhibin*-α	PhosH3 Ser10[a]	[77]
		SGK, *inhibin*-α	AcH3 Lys9 and/or Lys14[b]	
		SGK, *inhibin*-α, c-*fos*	Phos/AcH3 Ser10/Lys14	
Mouse C3H 10T1/2 fibroblasts	Arsenite	*MKP*1	PhosH3 Ser10[a]	[87]
		*MKP*1, c-*fos*	Phos/AcH3 Ser10/Lys14	
Yeast *Saccharomyces cerevisiae*	Inositol starvation	*INO*1	PhosH3 Ser10[a], Phos/AcH3 Ser10/Lys14, AcH3 Lys9 and/or Lys14[b]	[51]
Mouse P19 embryonal carcinoma cells	atRA	*RAR*-β2	PhosH3 Ser10[a]	[96]
Human primary dendritic cells	LPS	*IL*-6, *IL*-8, *MCP*-1 *IL*-12p40	PhosH3 Ser10[a]	[84]
Mouse Swiss 3T3 cells	sAn and TSA	*HDAC*1[c]	Phos/AcH3 Ser10/Lys14	[97]
Mouse B6.1 cells	IL-2	*HDAC*1[d]		
Human acute promyelocytic leukaemia NB4 cells	Arsenic trioxide	*CASPASE*-10[f]	Phos/AcH3 Ser10/Lys14	[88]
Human WI-38 fibroblasts	Arsenite	c-*fos*, c-*jun*	PhosH3 Ser10[a], Phos/AcH3 Ser10/Lys14	[26]
Human HeLa cells	Virus infection	*IFN*-β promoter[e]	AcH4 Lys8, Lys12, AcH3 Lys9, Lys14, PhosH3 Ser10[a]	[40]
Human glioblastoma T98G cells	TPA and serum	*Collagenase*[g]	PhosH3 Ser10, AcH3 Lys9 and/or Lys14[b], AcH4	[98]

Modifications tabulated here are those for which a stimulus-induced increase was observed. Other modifications may be present, but the levels at specific genes do not increase upon stimulation or were not assayed.
Abbreviations: EGF, epidermal growth factor; EAn, EGF/anisomycin; sAn, sub-inhibitory anisomycin; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; FSH, follicle-stimulating hormone; atRA, all-*trans*-retinoic acid; LPS, lipopolysaccharide; *SGK*, serum glucocorticoid kinase; *MKP*1, mitogen-activated protein kinase phosphatase; *IFN*, interferon; *IL*, interleukin; *MCP*-1, macrophage chemoattractant protein-1.
[a]Recognition by this PhosH3 Ser10 antibody is not affected by acetylation at Lys14.
[b]Recognition by this AcH3 Lys9 and/or Lys14 antibody is lost when serine 10 is phosphorylated [20]. This antibody has recently been shown to be largely specific for AcH3 Lys9 [83] although previous peptide competition experiments showed that the antibody was blocked in the presence of an AcH3 Lys14 peptide [19].
[c]HDAC1 is a late inducible gene. ChIP assays were after 3 h sAn and TSA (Trichostatin A) treatment.
[d]ChIP assays were after 16 h IL-2 treatment.
[e]IFN-β mRNA is first detected 6 h after virus infection. H3 and H4 modifications are first detected 2–3 h after virus infection.
[f]Peak induction for this gene was after 12 h treatment with arsenic trioxide. ChIP assays were after 48 h treatment.
[g]Methylation at H3 Lys4 was also observed at the collagenase promoter.

fection of CLS fibroblasts with RSK2 DNA restored the EGF-stimulated phosphorylation of histone H3 [70]. Mitotic H3 phosphorylation still occurred normally in these cells. Based on these experiments, RSK2 was proposed as the ligand-stimulated H3 kinase. In the light of more recent experiments, these conclusions, and indeed the use of CLS fibroblasts to derive kinase–substrate relationships, need to be re-examined. Firstly, the deficiency for mitogen-stimulated (EGF) CREB phosphorylation that was observed in CLS cells has not been observed in recent studies with other mitogens using mouse RSK2 knockout cells. In these cells platelet-derived growth factor- and insulin-like growth factor-1-stimulated CREB phosphorylation was unaffected indicating no requirement for RSK2 [71]. In addition, MSK1/2 has been identified as the major mitogen/stress-induced CREB kinase(s) from studies in MSK1 [72] and MSK1/2 knockout cells [73]. Furthermore, these authors were unable to reproduce the EGF-stimulated CREB phosphorylation defect previously reported in the CLS fibroblasts [73]. It is unclear why there is a difference in signalling to CREB and ATF1 between the RSK2 knockout and CLS cells. One possible explanation may be additional uncharacterised mutations in other kinases in the CLS cells, or that during their establishment in culture, other kinases in CLS fibroblasts have been affected. Similar problems have now arisen in respect of the reported histone H3 phosphorylation defect in CLS fibroblasts. Recent attempts to reproduce these experiments have failed; a completely normal histone H3 phosphorylation response was seen in CLS fibroblasts, compromising the major piece of reported evidence for RSK2/MAPKAP-K1b being the inducible histone H3 kinase [56].

7. MSK1/2

MSK1/2 were identified by homology searching using the MAPKAP-K1 N-terminal domain [74]. They are localised in the nucleus, consistent with roles in generating nuclear responses, and are activated by both the ERK and p38 MAP kinase pathways [74]. In vitro studies have shown that MSK1 phosphorylates H3 (and HMG-14) on the physiologically relevant sites and with a much higher efficiency than either RSK1 or RSK2. Further, the small molecule inhibitor H89 inhibits MSK1/2 activity in vitro and inhibits the nucleosomal response and IE gene induction elicited via both the ERK and p38 MAP kinase pathways in vivo at concentrations at which RSK1 and 2, MAP kinases and transcription factors are not affected [17]. From these studies MSK1 was identified as the best candidate kinase for the phosphorylation of H3 and HMG-14.

The most conclusive evidence for MSK1/2 as the H3/HMG-14 kinase has emerged very recently. Studies in MSK1/2 single- and double-knockout cells show that TPA- and anisomycin-induced (as well as EGF- and UV-induced) histone H3 (and HMG-14) phosphorylation is severely reduced or abolished, indicating that MSKs, particularly MSK2, are the major H3 (and HMG-14) kinases activated by both mitogens and stress [56]. The H3 phosphorylation defect is recoverable by reintroduction of MSK2 by transfection into these cells.

8. Stimulus-induced histone H3 kinases in other organisms

Homologues of the downstream kinases MSK and RSK have not been identified in *Saccharomyces cerevisiae*, although equivalents of the ERK and p38 MAP kinases are present. However, Snf1 has been identified as an H3 kinase responsible for the inducible H3 Ser10 phosphorylation observed at the activated INO1 gene in response to inositol deprivation in yeast [51]. In *Drosophila* the JIL-1 kinase has been identified as a homologue of MSK that localises specifically to the gene active interband regions of larval polytene chromosomes and phosphorylates histone H3 in vitro [75] but this may be involved in the maintenance of chromatin structure during interphase rather than with inducible H3 phosphorylation [76].

Protein kinase A (PKA) has also been reported to be a histone H3 kinase in rat ovarian granulosa cells [77]. H3 phosphorylation induced by follicle-stimulating hormone (FSH) was not inhibited by pre-treatment with a variety of inhibitors to various signalling pathways, including the ERK pathway inhibitor PD098059 and the p38 pathway inhibitor SB203580 [77]. A dependence on PKA was determined by the reduction in FSH-stimulated H3 phosphorylation when cells were pre-treated with the PKA inhibitor protein peptide PKI attached to the cell-permeabilising TAT peptide. PKA was also shown to phosphorylate H3 directly in vitro [77]. However, the original demonstration of PKA as an efficient kinase for histone H3 occurred during investigations on condensed mitotic chromosomes [42,78], now thought to be mediated in vivo by Aurora/Ipl kinases discussed above.

9. Does histone H3 phosphorylation direct its subsequent acetylation to produce phosphoacetylation?

From the increasing number of chromatin immunoprecipitation (ChIP) experiments with a variety of modification-specific histone antibodies, the picture of inducible H3 phosphorylation, acetylation and phosphoacetylation, targeted to (IE)/inducible gene chromatin that is emerging is one of a complex and dynamic pattern of multiple modifications. Present indications are that phosphorylation occurs at Ser10, and lysines 9 and 14 are both acetylated, probably simultaneously [19,20] (see Table 2). How both modifications are targeted to the same H3 tail and the functional interrelationship between them remains unclear and is another area of controversy in the literature. There are two possible mechanisms.

9.1. Synergistic coupled modifications

One possibility is that the two modifications are mechanistically linked so that phosphorylation of H3 directs its subsequent acetylation. This is the coupled and synergistic model that has been proposed to account for the EGF-stimulated phosphorylation (Ser10) and acetylation (Lys14) observed at the c-*fos* gene in mouse cells, [19] and the *INO*1 gene in yeast cells [51]. The experiments in mouse cells are discussed further below (Section 9.2). This model is based on the demonstration in vitro that several recombinant HAT proteins, including the prototypical Gcn5, preferentially acetylate an H3 peptide phosphorylated at Ser10 compared with the equivalent unmodified H3 tail peptide [19,79]. Furthermore, this preferential acetylation of the phosphopeptide, reportedly at Lys14, was lost in a Gcn5 mutant with Arg164 changed to alanine, a residue in close proximity to H3 Ser10 in the Gcn5/CoA/H3 peptide crystal structure [79]. In yeast, this sequential model has been proposed for the phosphorylation and acetylation observed at the *INO*1 gene, in response to inositol starvation,

with Snf1 as the H3 kinase [51]. Using Ser10 mutant strains, as well as Snf1- and Gcn5-deficient strains, Lys14 acetylation by Gcn5 was shown to require phosphorylation at Ser10 [51]. Whether Gcn5 is responsible for the acetylation at H3 Lys14 is not entirely clear. Surprisingly, ChIP data for a number of yeast promoters, including *INO*1, show that Gcn5 acetylates all sites of H3 except Lys14 [80]. These experiments were done using histone site-specific acetyl-lysine antibodies that were characterised by ChIP screening against specific lysine substitution mutations.

These studies have recently been advanced using a new technique that allows these assays to be performed not just on modified synthetic peptides, but on reconstituted nucleosomal arrays with predetermined histone tail modifications [81]. A peptide ligation strategy was used in which chemically synthesised histone H3 N-terminal tail peptides with or without phosphorylated Ser10 were ligated with the purified recombinant C-terminal domain of H3. Nucleosomal arrays were then reconstituted using octamers containing the ligated H3 molecules. These reconstituted arrays with phosphorylated histone H3 were tested in HAT assays using Gcn5 alone or in the native SAGA complex. Interestingly, while Gcn5 preferentially acetylated H3 in the nucleosomal arrays reconstituted with phosphorylated H3 in accord with studies described above [19,51,79], Gcn5 in the SAGA complex showed no preference for phosphorylated H3 over non-phosphorylated H3 in these arrays. Possible reasons for this difference could be that in the SAGA complex other subunits may influence the mode of binding to the H3 tail. Also, there may be additional contacts within the nucleosome, for example contacts with other histone tails, that are not possible with peptide substrates [81]. Using this peptide ligation strategy it should be possible to generate and analyse nucleosomal arrays containing single or multiple post-translational modifications, which should advance understanding of their possible functions.

9.2. Independent dynamic modifications

A distinct model envisages that phosphorylation and acetylation are independently targeted to the same nucleosomes by recruitment of the relevant enzyme complexes to regulatory elements in the gene as opposed to the H3 tail itself, and that the modifications can coincide to produce phosphoacetylation on H3 tails [20,31].

Work from our lab supports this second mechanism. Antibodies that recognise mutually exclusive combinations of modifications were used to show that nucleosomes from the same region of active IE gene chromatin exist in two forms at the same time, a larger highly acetylated population that is not phosphorylated and a smaller phosphoacetylated (Lys9/Ser10) population. The existence of these two separate populations indicates that prior phosphorylation is not obligately required for acetylation. Furthermore, in support of the 'synergistic and coupled' model, it was previously claimed that the inhibition of histone H3 phosphorylation using MAP kinase inhibitors resulted in an inhibition of acetylation at IE genes [19]. This has not proved reproducible despite being analysed in the same cell type; experiments in our lab using several stimuli and kinase inhibitors led to the opposite conclusion, namely that inducible H3 acetylation at IE genes was normal even when its phosphorylation was blocked [20]. This model has received further support recently; in MSK1/2 knockout cells, histone H3 phosphorylation at IE genes is severely reduced, whereas inducible acetylation at these genes is relatively unaffected [56]. A final piece of evidence in support of independent dynamic modifications comes from studies with TSA, which showed that acetylation is continuously turning over even in quiescent cells, indicating that HATs and HDACs are already operating at IE genes, even in the absence of MAP kinase activation or H3 phosphorylation. Hence, H3 phosphorylation is not required for targeted acetylation at these genes [20].

The overwhelming indication from these studies is that in mouse fibroblasts, phosphorylation and acetylation are independently delivered to histone H3 at IE genes and that these modifications are highly dynamic in nature and can coincide to give rise to phosphoacetylation at a subset of tails.

A point worth mentioning here is that consideration of antibody specificities, a major complication in these studies, is critical in the interpretation of all these experiments (discussed in [82]). For example, several studies have now shown that the widely used commercially available anti-acH3 Lys9/14 antibody is occluded by phosphorylation at Ser10 [20], a complication that affects some of the earlier studies using this antibody [51]. Furthermore, it is now shown to be largely specific for H3 acetylated at Lys9 and not Lys14 [83] although previous peptide competition experiments showed that this antibody was blocked in the presence of an acH3 Lys14 peptide [19].

10. MAP kinase-mediated acetylation of histones on inducible genes

While the ERK and p38 pathways are clearly responsible for delivering histone H3 phosphorylation, the nature of the signal that causes enhanced acetylation at the same histone tails is presently unclear. Intriguingly, a distinct MAP kinase cascade, the JNK/SAPK cascade (see Fig. 1), has been identified as a potential mediator of this histone acetylation at IE genes [35]. By microinjection experiments in serum-starved NIH3T3 cells these authors found that activation of the chromosomal c-*fos* gene or integrated serum response factor-controlled reporter genes by a constitutively active form of the small GTPase RhoA required activation of an additional signalling pathway (the JNK/SAPK pathway) that also induced H4 acetylation at these genes. Thus, distinct MAP kinase cascades may eventually turn out to converge on the same histone H3 tail to elicit their multiple modification during gene induction. This is an area of great current interest which should be resolved in the near future.

11. Possible roles of inducible H3 phosphoacetylation in (IE) gene transcription

Signal-induced phosphorylation of serine 10 and/or phosphoacetylation of histone H3 has now been observed in an increasing number of systems, documented in Table 2. Although a clear link between mitogen- or stress-inducible histone H3 phosphorylation and gene transcription (and histone H3 acetylation) is established, its precise molecular function remains unclear and represents the next major challenge in this area. When H3 (and HMG-14) phosphorylation is blocked either by the treatment of cells with H89 [17] or in MSK1/2 knockout cells [56] IE gene induction is not ablated but the efficiency of induction is affected, altering their char-

acteristic patterns of expression. This indicates that the nucleosomal response may not be essential for IE gene induction in the same way as transcription factor phosphorylation [28,31], but that it may influence the rate/profile of expression of these genes. One broad class of function would be that phosphorylation mediates changes in nucleosome and chromatin structure by disrupting/altering histone–DNA charge interactions facilitating easier access to the underlying DNA sequences by transcription factors. This has been suggested for a subset of stimulus-induced NF-κB-dependent cytokine and chemokine genes in which p38-induced H3 phosphorylation was proposed to enhance the accessibility of the NF-κB binding sites [84]. A second function, more aligned with the histone code hypothesis, is that the phosphoacetyl epitope on histone H3 at Ser10 (as well as on Ser6 of HMG-14) serves as a binding motif for recruitment of coactivator complexes such as HATs or chromatin remodelling complexes, in the same way as is already established for phosphorylated transcription factors at upstream promoter sequences [85,86].

The fact that inducible H3 phosphoacetylation is a tightly regulated process and occurs on only a small fraction of the nucleosomes associated with IE genes is now well established in the literature. Outstanding questions remain about the precise interplay of multiple modifications, the mechanisms by which the two enzyme systems are targeted to the same histone H3 tail and perhaps most importantly, their precise molecular role in the process of IE gene induction.

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