Cell Signaling and Transcriptional Regulation via Histone Phosphorylation

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Regulation of transcription involves a large number of histone lysine and arginine posttranslational modifications found marking gene promoters and gene bodies. Within histones there are abundant accessible serine/threonine/tyrosine residues for potential phosphorylation; however, few sites have been clearly documented with regard to actual modification, relevant physiological pathway, and cognate enzyme. In addition, kinases within signaling pathways are thought to be localized to the cytoplasm and thus not able to directly modify histones within chromatin in the nucleus. However, direct assays in the model eukaryote *Saccharomyces cerevisiae* via chromatin immunoprecipitation have placed numerous signaling kinases at promoters and within gene bodies. In addition, recent studies in mammalian cells of two signaling pathways place the terminal kinase within the nucleus or directly at genes, have identified histone phosphorylation sites, and furthermore, have uncovered potential mechanisms by which these histone phosphorylation sites activate transcription. These results lead to a gathering appreciation of the potential role of signal transduction kinase-mediated direct histone phosphorylation in regulating transcription.

HISTONE MODIFICATION

Protein phosphorylation is a dynamic posttranslational modification, providing a rapid and reversible physiological response to changing cellular conditions, such as DNA damage, nutritional stress, or altered metabolic state. Many signaling pathways involve cytoplasmic phosphorylation cascades, such as classic MAP kinase pathways signaled by growth factors and mitogens, and triggering the cell cycle. These pathways typically also regulate transcription, frequently via phosphorylation of transcription factors to alter their activity, including DNA-binding affinity, conformation, and interaction with other proteins. However, whether these phosphorylation cascades ultimately regulate transcription directly, via nuclear import of the terminal kinase in the pathway and modification of substrates bound to DNA, is only starting to be uncovered.

Chromatin-associated proteins, such as the major histone constituents, are modified by phosphorylation, largely on frequently occurring serine/threonine (S/T) residues, and also on tyrosine (Y) (Nowak and Corces 2004; Pérez-Cadahía et al. 2010). There are eight characterized phosphorylation sites on the core canonical histones H2A, H2B, H3, and H4, which have been linked to specific cognate kinases (Fig. 1A). These occur in several pathways including DNA damage, mitosis/meiosis, apoptosis, and nuclear hormone signaling (Clayton and Mahadevan 2003; Dyson et al. 2005; Carmona-Gutierrez and Madeo 2006; Vicent et al. 2006; van Attikum and Gasser 2009; Govin et al. 2010). Interestingly, there are numerous additional potential sites, including 27 serines and threonines in the exposed amino and carboxy-terminal tails plus the loops within the globular domains (Fig. 1B). In addition, there are exposed tyrosines, and there is little known regarding their potential phosphorylation.

Certain histone modifications are abundant, such as lysine modifications that mark genes on their regulatory and structural regions (Berger 2007; Kouzarides 2007; Smith and Shilatifard 2010). These include lysine acetylation (largely activating), lysine methylation (either activating or repressing), lysine ubiquitylation (either activating or repressing), lysine sumoylation (repressing), and arginine methylation (activating). The stability of these modifications is variable, with acetylation and ubiquitylation dynamic and transient, whereas methylation is stabler and longer lasting. These modifications are believed to mark most genes during activation and repression, as well as broader heterochromatic genomic regions.

In contrast, there is inherent difficulty in discovering modifications that occur only in inducible, transient, and highly specific pathways, because their abundance is likely to be low and they persist only briefly. Phosphorylation tends to fit this profile, and hence, the discovery and elucidation of function of specific histone phosphorylations, compared to the aforementioned modifications of lysine and arginine, have been revealed relatively slowly. This review focuses on evidence that histone phosphorylation may be a common feature of signal transduction pathways, in response to cell cycling or cellular stress. Thus, an interesting aspect of these findings is that terminal kinases in these pathways may lead directly to chromatin modulation at genes.

SIGNAL TRANSDUCTION INTO THE NUCLEUS VIA KINASE RECRUITMENT TO GENES

Interesting questions concern the kinases and phosphorylation substrates in the numerous and varied cellular signal transduction pathways (Edmunds and Mahadevan

Figure 1. (A) Histone phosphorylation sites and cognate enzymes on the core histones. Only eight phosphorylations have been characterized regarding precise residues, enzymes, and pathway. (B) Serine, threonine, and tyrosine residues within core histones. Residues localized in potentially accessible regions are numbered; these occur in amino/carboxy-terminal tails, or in probable loops and exposed regions within globular domains.

2004). These questions include whether the terminal kinase in these pathways (1) is nuclear-localized, (2) associates stably with genes, and (3) targets histones within chromatin as substrates. The enabling technical approach in the series of experiments that is described here is chromatin immunoprecipitation (ChIP) to examine direct and specific gene association of the enzymes and their substrates.

The homologous MAP kinases, yeast Hog1 and mammalian p38, provided initial clues that stress signaling kinases are nuclear and interact stably with chromatin (for review, see Edmunds and Mahadevan 2006). Hog1 responds to osmotic stress and translocates to promoters of genes that are regulated in that pathway to induce expression. The recruitment of Hog1 requires a DNA-bound transcription factor and protein-protein interactions between the DNA-bound factor and Hog1 kinase. Similarly, mammalian p38 associates stably with the enhancer region of muscle-specific genes during myogenic differentiation.

Spurred by these findings, a more systematic genome-wide study, using ChIP combined with microarray gene hybridization, was carried out to investigate a number of signaling kinases in yeast for potential association with chromatin around genes (Pokholok et al. 2006). Several signaling kinases, when inducibly activated, were found to associate directly with target genes genome-wide, and patterns of association across genes emerged. One pattern was typified by the MAPK Hog1, where the association was highest at the promoter and lower across the transcribed regions. In contrast, three MAP kinases in the pheromone signaling pathway exhibited coassociation genome-wide and showed a different gene association pattern, with weak signals over the promoter but much stronger signals over the transcribed gene.

These distinct patterns suggest that there may be several different mechanisms for recruitment of these kinases, including (1) transcription factor-kinase interaction at the promoter, (2) recruitment via the early elongation form of RNAP II, or (3) recruitment via the late elongation form of RNAP II. In addition, the patterns indicate that the resulting phosphorylation may also exert distinct downstream effects. For example, the substrate of the kinase

may regulate transcriptional initiation, early elongation, or later elongation of transcription.

These results indicate that association of signaling kinases with promoters and transcribed genes occurs frequently in yeast and further suggest that certain kinases may target a substrate(s) that is present throughout the gene. Key questions that emerge are whether (1) histones are substrates of these kinases within genes, (2) mammalian signaling kinases similarly commonly associate with genes, and (3) the modifications regulate transcription by association or by inhibiting association of a cognate protein. Two recent reports regarding mammalian signaling kinases indicate that histones are, indeed, a probable substrate at gene targets and provide insight into the mechanistic outcome of the phosphorylation.

JAK2 KINASE PHOSPHORYLATES HISTONE H3 TO INHIBIT HP1A ASSOCIATION WITH GENE PROMOTERS

The mammalian Jak2 (Janus Kinase 2) tyrosine kinase initiates a signaling pathway involved in cell cycle progression and apoptosis; disruptions of this pathway are oncogenic. Jak2 was thought to be constitutively cytoplasmic and signaled via transcription factors to activate transcription. However, a recent study reexamined Jak2 localization in hematopoietic cells using immunofluorescence and cell fractionation and found a nuclear portion of Jak2 (Dawson et al. 2009). Both recombinant and hematopoietic cell-derived Jak2 phosphorylated histone H3, and, of three tyrosines in H3, amino-terminal Y41 was found to be the key substrate *in vitro*. Importantly, an antibody developed for H3Y41ph detected the site *in vivo*, and an increase in the H3Y41ph signal was found following stimulation of the Jak2 pathway using cytokines or growth factors, or after transfection of Jak2, whereas a specific Jak2 inhibitor strongly decreased the histone signal.

A novel regulatory mechanism of H3Y41ph was suggested by previous findings in *Drosophila* that the Jak pathway altered heterochromatin by disrupting heterochromatic protein, HP1 (Shi et al. 2006). HP1 binds to H3 K9me3 via

its chromodomain and recruits several chromatin repressive complexes that down-regulate transcription (Kouzarides 2007). Therefore, based on the Jak pathway/HP1 link, HP1 was tested for binding to the Y41 region of H3 in mammalian cells. HP1 was found to associate, via a chromodomain-like domain, with an H3 peptide corresponding to unmodified Y41, whereas Y41ph within the peptide disrupted this binding. Of physiological relevance, HP1 binding within the nucleus to heterochromatic speckles was competed by a peptide corresponding to unmodified H3 Y41, but not by H3 Y41ph, and, using a biochemical approach, the fraction of chromatin-bound HP1 was reduced following pharmacological inhibition of Jak2.

Indeed, pharmacological inhibition of Jak2 led to inhibition of genes previously noted to be transcriptional targets of Jak2. Detailed characterization of one gene using ChIP showed that down-regulation of Jak2 caused decreased H3 Y41 phosphorylation and increased HP1 binding. These results suggest that induction of the Jak pathway induces J3 Y41 phosphorylation directly at genes to dislodge repressive HP1 and hence activate transcription (Fig. 2A). This pathway appears to be important in both hematopoietic cells and in embryonic stem cells (Griffiths et al. 2011).

AMPK IS RECRUITED BY P53 TO PHOSPHORYLATE H2B AT S36 IN GENE PROMOTERS AND GENE BODIES

A second recent study focusing on a mammalian signaling kinase, AMPK, uncovered a nuclear, chromatin-based role for direct transcriptional activation by this enzyme (Bungard et al. 2010). AMPK responds to nutrient stress, such as low glucose concentration and a high AMP/ATP ratio in the cell, to slow metabolism and increase catabolism. AMPK is cytoplasmic and has previously been thought to signal via intermediary substrates to eventually regulate transcription.

This study revealed a metabolic, and DNA-damage, inducible association of AMPK with target genes. Thus, apparently, AMPK responds generally to stress, beyond metabolic stress, to arrest the cell cycle and trigger survival. Interestingly this pathway is a classic p53-mediated

transcriptional activation pathway. Hence, transcription of p53 target genes, such as the cell cycle arrest gene p21, are induced by low glucose or DNA damage and are dependent on AMPK for activation; furthermore, AMPK is directly recruited to the promoters. Surprisingly, p53 associates with AMPK and is required for AMPK recruitment.

As in the case of Jak2 described above, because of the direct association of AMPK with genes, a possible substrate would be a histone. Direct comparison of activity of AMPK derived from cells under stress, compared to non-stressed cells, showed specific histone H2B phosphorylation *in vitro*, and H2B S36 was identified as the key substrate site. An antibody generated to this site showed global increase in H2B S36ph in various stress conditions, as well as an increase in local ChIP signal at target genes such as p21, depending on AMPK. Mapping of ChIP signals for AMPK and H2B S36ph showed induced signals in stress conditions, and colocalization of AMPK/S36ph, not only at the promoter, but also across the transcribed region of target genes.

To determine the importance of the histone phosphorylation for the stress responses, clonal cell lines were derived ectopically expressing wild-type H2B or a substitution mutation H2B S36A and tested for stress response. The mutant lines showed reduced expression of p53 target genes, such as p21, and impaired ability to survive stress. The affected genes showed a reduction of RNA polymerase II, especially to distal portions of the gene. These data indicate that, in response to metabolic and genotoxic stress, p53 recruits AMPK to key target survival genes, to phosphorylate H2B, leading to recruitment of RNA polymerase II and transcription (Fig. 2B).

CONCLUSIONS AND FUTURE DIRECTIONS

The major conclusions that emerge from these experiments concern the ultimate destination of kinase cascades in the cell, and the identity and mechanisms of the substrate. The central idea is that signaling kinases may, in general, have a direct transcriptional function, in addition to modifying transcription factors in the cytoplasm for import into the nucleus. Hence, the terminal kinase at the

Figure 2. (A) Schematic pathway of proposed Jak2-mediated histone phosphorylation at H3 Y41. The mechanism of gene activation via Y41ph is inhibition of repressive HP1 α binding to this region of H3. This occurs over the promoter region of the gene. (B) Schematic pathway of proposed AMPK-mediated histone phosphorylation at H2B S36. AMPK is recruited to the gene by protein-protein interaction with the transcription factor bound to DNA upstream. AMPK and H2B S36ph extend over the transcribed region of the gene. The mechanism of gene activation via S36ph is to increase RNA polymerase II association over the gene.

end of these cascades is itself imported into the nucleus, associates with genes, and phosphorylates histones, which leads to downstream effects to alter transcription initiation or elongation by RNA polymerase II (Fig. 2).

There are numerous intriguing questions and key extensions of these observations. First, it will be interesting to determine how the kinases (and their complexes) associate with the transcribed region of genes—is it random association or a targeted event? Hog1 and AMPK indicate that association of the enzyme can be targeted, as each appears to be recruited by direct association with a DNA-bound activator. In addition, it is possible that these kinases associate with open chromatin across genes as nucleosomes are disassembled and the RNAP II complex moves along transcribing the gene.

In the case of Jak2 and AMPK, the location of the substrate residue is in the first helix of H3 or H2B, and it may be that these residues are only partially accessible. Hence, a histone chaperone may be required to assist the kinase to reach its substrate residue within the nucleosome.

A second important area of inquiry is the mechanistic effect of the histone phosphorylation. In the examples presented, the outcome is activation of expression of genes that respond to the altered condition. To initiate transcription, one key function of the phosphorylation may be to remove repressive proteins, as in the case of Jak2/Y41ph and removal of HP1. It is equally possible that binding of DNA-bound repressors or corepressors could be inhibited by phosphorylation. In addition, direct positive functions in transcription are suggested by the recruitment via the activator p53, and location of AMPK both at the promoter and across the transcribed region of the gene. For example, the phosphorylation may help to recruit initiation or elongation factors to the promoter and across the gene, which could then promote RNA polymerase II association with the template.

The most provocative question regards the generality of these observations. It is clear that there are many potential histone phosphorylation sites, given the large number of accessible Ser/Thr/Tyr residues, and thus the challenge for the future is to determine how many of these sites are actually used in signal transduction pathways.

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