REVIEW

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Histone tyrosine phosphorylation comes of age

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Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; BRCT, BRCA1 C-terminus; CHEK1, checkpoint kinase 1; CHEK2, checkpoint kinase 2; DSB, double strand break; EYA, eyes absent; F, phenylalanine; JAK2, Janus kinase 2; JNK1, c-Jun N-terminal protein kinase 1; MDC1, mediator of DNA damage checkpoint protein 1; S, serine; Sir, silence information regulator; T, threonine; WSTF, Williams-Beuren syndrome transcription factor; WICH complex, WSTF-ISWI ATP-dependent chromatin-remodeling complex; Y, tyrosine

Histones were discovered over a century ago and have since been found to be the most extensively post-translationally modified proteins, although tyrosine phosphorylation of histones had remained elusive until recently. The year 2009 proved to be a landmark year for histone tyrosine (Y) phosphorylation as five research groups independently discovered this modification. Three groups describe phosphorylation of Y142 in the variant histone H2A.X, where it may be involved in the cellular decision making process to either undergo DNA repair or apoptosis in response to DNA damage. Further, one group suggests that phosphorylation of histone H3 on Y99 is crucial for its regulated proteolysis in yeast, while another found that Y41 phosphorylation modulates chromatin architecture and oncogenesis in mammalian cells. These pioneering studies provide the initial conceptual framework for further analyses of the diverse roles of tyrosine phosphorylation on different histones, with far reaching implications for human health and disease.

Introduction

Histones are essential eukaryotic proteins that package the genetic material (DNA) inside the cell nucleus.[1] Two molecules of each of four core histones (H2A, H2B, H3 and H4) form an octameric histone core around which 147 bp of DNA is wrapped to form a nucleosome, the fundamental repeating unit of chromatin.[2] The classic viewpoint that histones serve as mere structural components of chromatin has been substantially modified over the past two decades largely due to discovery of a plethora of post-translational modifications on histones that play a regulatory role in almost all DNA transactions. Histones are extensively modified proteins and the constituent amino acid residues of each histone can undergo multi-site post-translational modifications, as well as multiple modifications on the same residue. The major core histone modifications known so far are acetylation and ubiquitylation on lysine residues, methylation on lysine and arginine residues and phosphorylation on serine (S) and threonine (T) residues.[3] These extensively modified histone molecules play a crucial role in regulating access to the genetic information contained in the DNA.[4] Cross talk between these multiple modifications has been documented frequently, although the regulation of these modifications is still poorly understood.[5] How the multiple histone modifications carry out their regulatory roles under physiological conditions has been an area of extensive research over the past two decades. Chromosomal histones are phosphorylated on serine and threonine residues for several processes, ranging from the control of gene expression (H3 S10 and T11), mitosis (H3 S10 and S28), apoptosis (H2B S10), meiosis and the DNA damage response (H4 S1 and H2A.X S139).[3,6-9] Although tyrosine (Y) phosphorylation of proteins as a key mechanism for signal transduction was discovered over three decades ago,[10] tyrosine phosphorylation of histones had remained elusive until very recently. The year 2009 proved to be a watershed year in histone tyrosine phosphorylation as five research papers from different groups were published almost simultaneously describing histone tyrosine phosphorylation.[11-15] While three of these papers uncovered tyrosine phosphorylation on the variant histone H2A.X, the other two papers described tyrosine phosphorylation of core histone H3. These phosphorylation events have been found to be important for the cellular response to DNA damage (H2A.X Y142),[11-13] histone turnover (H3 Y99)[14] and chromatin architecture and oncogenesis (H3 Y41).[15] These discoveries provide a novel perspective on the role of histone tyrosine phosphorylation in chromatin structure and function, genome maintenance and carcinogenesis. This review will focus on the discovery of the different histone tyrosine phosphorylation revealed recently, with particular emphasis on their implications for human health and disease, as well as future research in the area of histone tyrosine phosphorylation.

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Tyrosine Phosphorylation

Reversible protein phosphorylation has emerged as one of the most important post-translational modifications involved in both the turning on and off as well as the fine tuning of biological processes.[16] Almost all aspects of cellular physiology are known to be

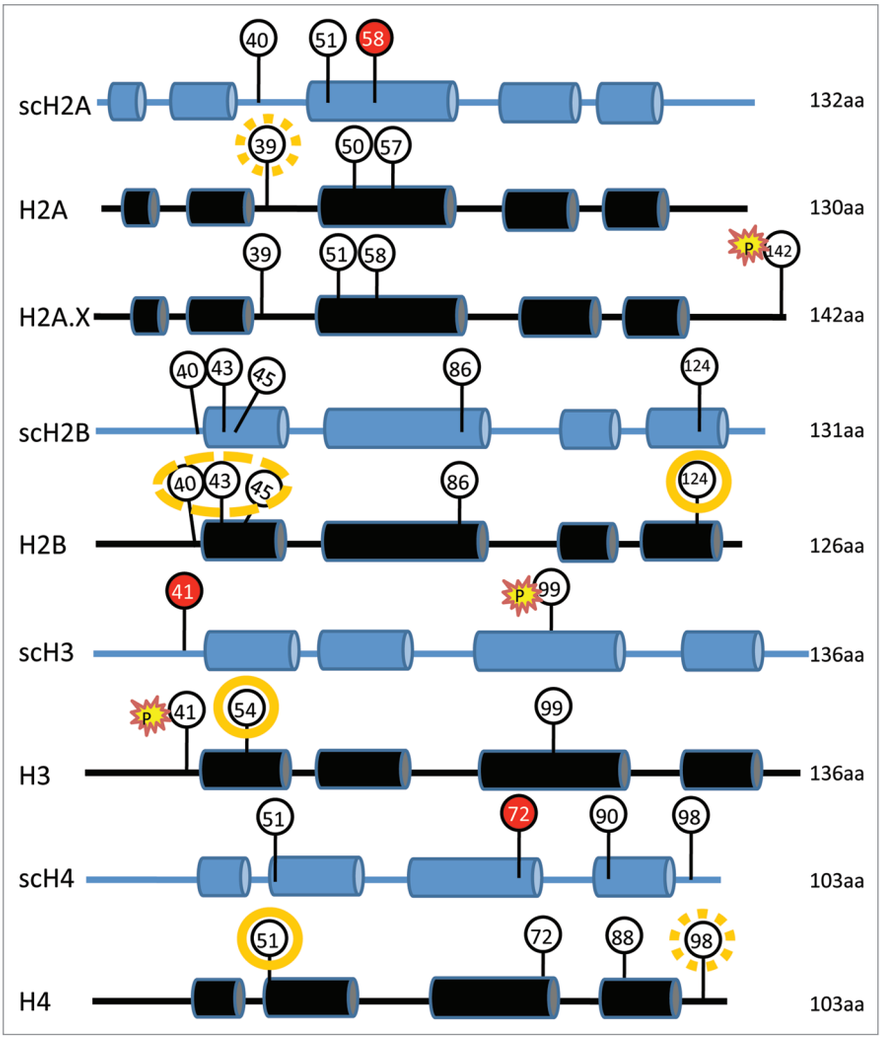

Figure 1. Tyrosine residues are highly conserved between budding yeast and mammalian core histones. The four canonical core histone proteins from the budding yeast *Saccharomyces cerevisiae* are indicated by the prefix "Sc" and denoted in blue. The mammalian core histones as well as the mammalian variant histone H2A.X are shown in black. The number of amino acid (aa) residues in each core histone is indicated on the right. The location of the α-helices in the secondary structure of the histone proteins is indicated by cylinders. Tyrosine residues are shown as balloons and the tyrosine residues essential for viability in budding yeast histones are indicated by red balloons. Tyrosines in mammalian histones have not yet been evaluated to determine the residues essential for viability. Note the high degree of conservation of the location as well as the spacing of all but one tyrosine residue between budding yeast and mammalian core histones (H3 Y54 being the exception). Tyrosine residues that have recently been shown to be phosphorylated in vivo are marked by yellow "explosion" signs and the letter "P." Additional tyrosine residues that are predicted to be reasonably accessible in the nucleosomal context under certain conditions and can be potentially phosphorylated in vivo are indicated by a yellow halo only on the mammalian histones for clarity, but are likely to be just as applicable to the yeast histones (solid yellow halo indicates higher probability of phosphorylation, while a dashed yellow halo indicates lower probability of phosphorylation).

regulated by the addition of phosphate groups to certain amino acid residues by enzymes known as protein kinases, or by their removal by enzymes known as phosphatases. Not surprisingly, the kinases and phosphatases discovered so far are known to regulate signaling cascades related to cell cycle progression, DNA replication, transcription, chromatin remodeling, translation, differentiation, cellular movement, cytoskeleton arrangements, intercellular communications, stress response, apoptosis and neurological functions.[17] Proteins can be phosphorylated on different amino acid residues, most commonly serines and threonines in eukaryotes. Just over three decades ago, Tony Hunter and colleagues demonstrated for the first time that tyrosine residues can also be phosphorylated.[10] Since then, tyrosine phosphorylation has been implicated in a wide range of signal transduction pathways essential for the proper control of several cellular activities, most importantly differentiation, cell growth and proliferation.[18] This reversible modification is added by protein tyrosine kinases and is removed predominantly by protein tyrosine phosphatases. The relative abundance of serine, threonine and tyrosine phosphorylation in the human phosphoproteome has been estimated to be 86.4, 11.8 and 1.8%, respectively.[19] Although tyrosine phosphorylation is relatively rare, it plays critical roles in human health and disease. More than half of the known tyrosine kinases have been implicated in cancer either through mutation or silencing or overexpression.[20]

Tyrosine Residues in Core Histones

All four core histones are very highly conserved proteins and the tyrosine residues in histones are amongst the most conserved residues in these proteins. Budding yeast H2A, H2B, H3 and H4 have 3, 5, 2 and 4 tyrosine residues respectively for a total of 14 tyrosine residues (**Fig. 1**), of which H2A Y58, H3 Y41 and H4 Y72 have been shown to be essential for survival using mutational analyses.[21,22] Similarly, a set of canonical mammalian core histones have 15 tyrosine residues in total, with H3 having an additional tyrosine residue (Y54, **Fig. 1**). Apart from being potentially phosphorylated, tyrosine side chains can form hydrogen bonds through their hydroxyl group, as well as participate in ring-stacking interactions with other aromatic rings thereby stabilizing both intra- and inter-molecular interactions.[23] The high degree of conservation of tyrosine residues suggests that there was strong selective pressure on them to remain unchanged during evolution and points to potentially crucial roles played by them in chromatin structure and function. Not surprisingly, the tyrosine residues in histones had been under intense scrutiny early on in the study of nucleosome structure. In fact, the initial efforts to elucidate the structure of the

nucleosome primarily involved biophysical studies of the accessibility of different histone tyrosines, which allowed their positions within the nucleosome to be inferred indirectly. As early as 1978 it was suggested that the tyrosines in histones may be involved in histone-histone interactions within the octamer.[24] Following this, there was a reasonably sustained effort to obtain a rough structure of the nucleosome using biophysical tools by studying the reactivity of the conserved tyrosine residues in histones under different conditions, which resulted in the generation of a wealth of data on nucleosome structure.[25-32] Several of these studies used crosslinking with ultraviolet (UV) light to probe the function of histone tyrosines in histone-histone interactions,[25,32] while others used differential UV absorption by the tyrosine residues in histones under different conditions.[30] Circular dichroism and measurement of changes in the intrinsic fluorescence of tyrosine residues in histones were also used as an indirect read out of their microenvironment within the nucleosome.[28-29] Additionally, some groups took advantage of the ease and specificity of imidazole spin labeling[27] or radioiodination[26] of tyrosine residues to probe their accessibility in denatured versus folded histones, as well as their accessibility within the nucleosome. The bulky iodination of tyrosine residues also allowed the effects of this modification on nucleosome reconstitution and nucleosome stability to be measured.[31] Together, these studies revealed that several tyrosine residues in the histone octamer are inaccessible and are likely to be involved in histone-histone interactions.[25-28,31,32] As such, iodination of these residues resulted in a reduction in nucleosome stability, although there was no measurable defect in the ability of these modified histones to be reconstituted into nucleosomes.[31]

Once low resolution crystal structures of the nucleosome and histone octamer started becoming available,[33-35] biophysical studies of the histone tyrosines slowed down considerably and finally came to an end with Alfred Zweidler's exhaustive mapping study of the 15 tyrosine residues in calf-thymus core histones on the basis of their reactivity with *p*-nitrobenzenesulfonyl fluoride (NBSF).[36] Zweidler showed that while all the tyrosines on individually isolated histones were accessible, only two of the 15 core histone tyrosines, mainly H2A Y121 and to a lesser extent H3 Y54, were accessible in native chromatin. However, additional tyrosines became accessible as the nucleosomal structure unraveled at higher ionic strengths up to 800 mM (H2B Y37, -Y40, -Y42 and H3 Y41), suggesting that these could potentially make contacts with DNA. The majority of the inaccessible tyrosines are located either at the H2A-H2B dimer, $(H3\text{-}H4)_2$ tetramer interface (H2B Y83 and H4 Y72, -Y88, -Y98), or deep in the α-helices involved in the H2A-H2B and H3-H4 dimerization surfaces forming the "handshake" motifs (H2A Y50, -Y57 and H3 Y99). Mutational analysis of histone H4 tyrosines in the budding yeast confirmed the tyrosine residues importance for the histone-histone contacts at the dimer-tetramer interface in vivo.[37] Finally, the high resolution crystal structure of the nucleosome[38] and its subsequent refinements[2,39,40] allowed the unambiguous determination of the location of histone tyrosines within the nucleosome and their interactions with neighboring groups in either DNA or histones in the nucleosome. The nucleosome crystal structure did confirm much of the conclusions derived from the biophysical and mutational studies, although few tyrosine contacts with DNA were observed (for example, H3 Y41). Hence, although the structural studies involving the histone tyrosines spanned over three decades and contributed tremendously towards our understanding of the nucleosome structure, they failed to provide a complete picture of the role of the tyrosine residues in histones that did not appear to make significant contacts with DNA or other histones but were, nevertheless, highly conserved, particularly in higher eukaryotes (for example, H4 Y51 and H3 Y54). It is possible that these tyrosines are playing an important role in the structural integrity of the N α-helix in their respective histones (**Fig. 1**), although mutational analysis of H4 Y51 does not support an essential role for this residue.[21,22] Another possibility is that these tyrosines may be undergoing phosphorylation, which in turn could be playing important, but not essential, roles in chromatin structure and function. Additional tyrosine residues that can be ascribed specific structural roles based on the crystal structure of the nucleosome may also be accessible for phosphorylation, at least under certain conditions (H2A Y39, H2B Y121, H3 Y41, -Y54 and H4 Y51, -Y98). Also, it is possible for the individual newly synthesized core histones to acquire phosphorylation on any of their tyrosines prior to their dimerization and incorporation into the nucleosome. Interestingly, tyrosine phosphorylation of proteins was discovered[10] around the time when intensive biophysical studies involving histone tyrosines were starting. However, in the absence of modern reagents such as modification specific antibodies and sophisticated tools such as mass spectrometry to detect post-translational modifications on histones, it would have been hard to study tyrosine phosphorylation in histones. Hence, three decades would pass following the discovery of protein tyrosine phosphorylation before histone tyrosine phosphorylation was reported.

Life or Death Decisions: Reciprocal Regulation of DNA Repair and Apoptosis by Phosphorylation of Tyrosine 142 on Histone Variant H2A.X

Since the early 1990s, the exhaustive ongoing scrutiny of histone modifications has revealed a wide array of post-translational histone modifications with diverse functions. With the development of sensitive modification specific antibodies and the advent of mass spectrometry to detect post-translational protein modifications, it was just a matter of time before researchers focused on the tyrosine residues in histones once again. In fact, four of the five publications describing tyrosine phosphorylation of histones utilized modification specific antibodies for their studies.[11-13,15] Additionally, one publication used mass spectrometry to identify the tyrosine kinase responsible for the modification.[11] However, apart from the right technology, the discovery of histone tyrosine phosphorylation also depended on the investigators asking the right functional questions of fundamental significance to cell physiology, and this aspect is highlighted by nearly all the publications describing this modification to date.

Apart from canonical core histone proteins, all eukaryotes have non-allelic primary sequence variant histones, some of

which have been shown to have specific functions.[1,41,42] Of the core histones, histone H2A has the largest number of variants, of which histone H2A.X is a relatively minor variant (comprising of ~10% of total H2A in the cell), but has been extensively studied.[42] The renewed focus on histone tyrosines was triggered in part by the discovery several years ago that DNA double strand breaks (DSBs) led to the phosphorylation of the variant histone H2A.X at a C-terminal serine (S139 in humans and S129 in budding yeast H2A) primarily by the ATM (ataxia telangiectasia mutated) kinase over large chromatin domains (~2 Mb in human cells and ~20–50 Kb in budding yeast).[43-45] This phospho-H2A.X (also known as γH2A.X) was important for the recruitment of multiple DNA repair factors via specific recognition of the C-terminus of γH2A.X by MDC1 (Mediator of DNA damage checkpoint protein 1), which ultimately results in the formation of a macromolecular protein assembly of repair complexes that can be readily visualized by microscopy.[46-48] Exactly how the phosphorylation of γH2A.X is regulated upon DNA damage was unclear until recently. Peptide library screening and the crystal structure of the γH2A.X-MDC1 BRCT domain complex suggested that tyrosine 142 residue (Y142) of γH2A.X, which is the terminal residue in this protein, may be regulating the binding of MDC1 to γH2A.X.[49,50] This prompted the Allis group to investigate if the H2A.X Y142 residue was phosphorylated in vivo.[11] They discovered that Y142 is indeed constitutively phosphorylated under normal physiological conditions and becomes dephosphorylated upon DNA damage. Further, they presented evidence that this phosphorylation was carried out by WSTF (Williams-Beuren Syndrome transcription factor), which is a component of WICH complex (WSTF-ISWI ATP-dependent chromatin-remodeling complex) and has intrinsic tyrosine kinase activity by virtue of its N-terminal domain, which lacked any homology to known kinase domains. Following DNA damage, WSTF was found to dissociate from chromatin resulting in a decrease in Y142 phosphorylation, which in turn seemed to promote the maintenance of phosphorylation at S139 as well as the γH2A.X dependent recruitment of MDC1 and other repair complexes.

Two additional groups[12,13] independently described the phosphorylation of H2A.X Y142, focusing on the important regulatory roles played by the phosphatases that remove this modification. Cook et al.[12] found that during embryonic development of mouse kidney H2A.X Y142 phosphorylation was reversible. In addition, they identified EYA (named after the Drosophila mutant "eyes absent") as the main tyrosine phosphatase responsible for this. Mammalian cells have four related EYA enzymes, EYA1-4, with EYA1 and EYA3 possessing significant level of phosphatase activity for H2A.X Y142. In agreement with the observations of Xiao et al.,[11] these authors found an EYA dependent reduction of Y142 phosphorylation following DNA damage. Similar findings were published by Krishnan et al.[13] implicating EYA as the tyrosine phosphatase responsible for dephosphorylation at Y142. Further, Cook et al.[12] demonstrated that checkpoint kinases ATM and ATR (Ataxia telangiectasia and Rad3 related) were likely to be phosphorylating EYA3 at S219 following DNA damage to facilitate its interaction with γH2A.X. Mutation of S219 residue in EYA3 abolished its recruitment to γH2A.X repair foci. Together these studies suggest that the balance between the opposing activities of WSTF and EYA will be the crucial determinant of the outcome of DNA damage response, since H2A.X S139 phosphorylation seems to be dependent on the phosphorylation status of Y142.[11-13] One of the interesting observations of Cook et al.[12] was that the doubly phosphorylated C-terminal H2A.X peptide did not bind to MDC1, indicating that Y142 dephosphorylation was required for γH2A.X accumulation. Instead, this peptide was bound by apoptosis inducing protein kinase JNK1 (c-Jun N-terminal protein kinase 1). This finding hints at the idea that the phosphorylation status of Y142 may serve as an important determinant of cell fate after DNA damage. Quite possibly, when low amounts of damage occur and repair is feasible, phospho-H2A.X Y142 is dephosphorylated, while S139 phosphorylation accumulates and leads to the formation of repair foci by recruiting repair factors such as MDC1. Under extensive damage conditions, phospho-H2A.X Y142 persists, while phospho-S139 is attenuated and this leads to the recruitment of JNK1 leading to apoptosis. This hypothesis is supported by increase in JNK1-H2A.X interaction when cells were exposed to high doses of radiation.[12]

The reciprocal regulation of DNA repair and apoptosis by the reversible phosphorylation of H2A.X Y142 is a very attractive hypothesis to explain how the cell selects the most appropriate response following DNA damage. However, this hypothesis raises many new questions and the finer details of such regulation would need to be worked out. For example, phosphorylation of S139 is a rapid event following DSB and reaches maximal levels within minutes, while dephosphorylation of Y142 is a relatively slow process and occurs over several hours. Since Y142 is constitutively phosphorylated under normal conditions, this implies that the C-terminus of H2A.X will be doubly phosphorylated at S139 and Y142 early on in response to DSBs, irrespective of the extent of damage. As predicted by the findings of Cook et al.[12] the doubly phosphorylated C-terminus of H2A.X in this scenario should promote the binding of JNK1 and activation of the apoptotic pathway even for a single DSB. However, this clearly does not happen in response to easily repaired DSBs, although the underlying reasons for this are unclear. An in depth commentary on this topic is available elsewhere.[51]

Regulation of Histone Proteolysis by Tyrosine Phosphorylation

Histones are essential for viability as they package the DNA to fit it in the nucleus and, in doing so, they also regulate access to the genetic information contained within the DNA.[4] Hence, all transactions involving DNA are likely to be affected by histone proteins levels.[52] Cells normally accumulate excess histones only transiently either when DNA replication slows down at the end of S-phase or when DNA damage occurs during S-phase.[53,54] When present in excess, the positively charged histones can potentially "stick" non-specifically to negatively charged molecules in the cell, including DNA and thereby adversely affect processes that require access to DNA.[55] Not surprisingly, persistent elevation

in histone protein levels result in sensitivity to DNA damaging agents and genomic instability in the form of enhanced chromosome loss in the budding yeast *Saccharomyces cerevisiae*.[53,56] To avoid the deleterious consequences of excess histone accumulation, their levels are tightly regulated in part by transcriptional and posttranscriptional mechanisms.[57-59]

Post-translational regulation of many cellular proteins takes place via a cascade of reactions initiated by phosphorylation of the substrate proteins, followed by their polyubiquitylation by the ubiquitin system and subsequent degradation by the multisubunit, multi-functional protease known as the proteasome.[60-62] Histone H2A was identified as the first ubiquitylated protein over three decades ago[63] and post-translational regulation of proteins via degradation by the ubiquitin-proteasome system was elucidated by the early 1990s.[64] However, evidence for polyubiquitylation of histones and their subsequent degradation remained elusive until recently. This is not surprising given that histones are generally considered to be extremely stable proteins with half-lives estimated to be in the range of several months.[65-67] This high metabolic stability is likely important for histones to fulfill their roles in the stable inheritance of epigenetic states through cell division. However, these long half-lives are likely to reflect the contribution of chromatin bound histones, as it has been shown that non-chromatin bound histones are unstable and are rapidly degraded with a half-life of around 30 min in the budding yeast.[53] This degradation of non-chromatin associated histones required the DNA damage checkpoint kinase Rad53 as well as the ubiquitin-proteasome system.[14] Intriguingly, Rad53 has been reported to be a dual specificity kinase that can phosphorylate both serine/threonine as well as tyrosine residues in vitro.[68]

The degradation of excess histones can be considered as a case of conditional degradation of proteins that are otherwise designed to be metabolically stable. Alternatively, it is also possible that all new histones that are not incorporated into chromatin are intrinsically unstable by default and they only become stabilized once properly packaged into nucleosomes. Such a scenario may involve some degradation-promoting modifications uniquely carried by all the new histones that are removed following histone deposition into chromosomes. Conceivably, either of the aforementioned possibilities could be achieved by marking excess histones with modifications that signal degradation and are never found in nucleosomal histones. Using a combination of cutting-edge and classic biochemistry, yeast genetics and site-directed mutagenesis, Singh et al.[14] showed that excess histones bound to Rad53 kinase are extensively phosphorylated, and at least a subset of the modification sites in excess histone H3 are tyrosine residues. Further analysis revealed that tyrosine 99 residue of H3 was crucial for the ubiquitylation and degradation of this histone, presumably due to phosphorylation by Rad53 at that site.[14] Because some of the tyrosine residues on histones are buried within the nucleosome,[2,36-40] it seems plausible that at least those residues may only be accessible to a kinase in the context of excess histones. This appears to be the case for tyrosine 99 of histone H3, which lays buried in the nucleosome[39] and is not accessible for phosphorylation in a nucleosomal context. In vivo, newly synthesized histones associate as either H2A-H2B or H3-H4 dimers prior to their incorporation into chromatin.[69,70] In fact, the H3 Y99 residue is located at the interface of the H3-H4 heterodimer and appears to stabilize the H3-H4 heterodimer via ring stacking interaction with the phenylalanine 61 (F61) residue of histone H4. This strongly implies that H3 Y99 can only be phosphorylated when it is not in association with histone H4 and that phosphorylation of Y99 may even disrupt the ring stacking interaction and preclude H3-H4 dimer formation.[14] Support for this idea comes from in vitro chromatin assembly reactions using the Xenopus egg extract system with mutant histones where a mutant H3 carrying glycine (G) residues at positions 97–100 showed a greater than 50% loss in its ability to be assembled into chromatin.[71] These findings would be consistent with a simple mechanism that ensures that only the non-chromatin bound excess histone H3 is specifically targeted for degradation via phosphorylation on Y99, while the nucleosomal H3 is spared from any inadvertent degradation. Rad53 associates with excess histone H3 and presumably phosphorylates it, thereby preventing its heterodimerization and incorporation into chromatin, with proteasomal degradation being its sole option.

Since newly synthesized histones exist as either H2A-H2B or H3-H4 dimers in vivo prior to their assembly into nucleosomes,[69,70] excess histones that accumulate as replication slows down are likely to exist predominantly as dimers, rather than individual histones. Thus, it is conceivable that the Rad53-dependent degradation system may have evolved the ability to simultaneously degrade both subunits in either H3-H4 or H2A-H2B dimers, even when only one partner is marked by phosphorylation (that is, the degradation signal is present in "trans"). This would act as a failsafe mechanism to ensure that individual histones that are each capable of associating inappropriately with the negatively charged DNA and interfering with DNA metabolism[55] are never released from the Rad53 complex. In fact, such trans signals for degradation of oligomeric proteins by the ubiquitin-proteasome system have been previously reported. For instance, subunits of the β-galactosidase tetramer that lack N-terminal degron signals can be degraded through recognition of degrons in the other subunits, provided that they contain a lysine for ubiquitin attachment.[72]

Due to the cytotoxicity of excess histones,[55] cells have to rapidly eliminate any histones that have not been incorporated in the chromatin. Compared to just two copies of each core histone gene in the budding yeast,[57] human cells have about two dozen copies of each core histone gene[41] and, as such, human cells are even more likely to face the adverse effects of excess histone accumulation. The tumor suppressor CHEK2 (checkpoint kinase 2)[73] is the human counterpart of the Rad53 kinase that controls histone proteolysis in budding yeast. Further, CHEK1 is an essential kinase closely related to human CHEK2; they exhibit similar and often overlapping substrate specificities.[74] Interestingly, CHEK1 has been shown to phosphorylate nucleosomal H3 T11 in vivo,[8,9] hinting at the possibility that histones may be an important class of substrates for CHEK1/CHEK2 in human cells. Future studies will determine if human cells too have a pathway for regulated histone proteolysis that is perhaps initiated by the phosphorylation of specific histone tyrosines or

other residues by CHEK2 or CHEK1, analogous to the situation in the budding yeast. Hence, regulated histone proteolysis may turn out to be extremely important for the maintenance of genomic stability, cell survival and prevention of diseases such as cancer in humans. Additionally, it was recently reported that extracellular histones are one of the major effectors of cell death during sepsis,[75] which is the tenth leading cause of death in the American population. It is also possible that excess or extracellular histones play a role in the development of auto-antibodies against histones in certain autoimmune diseases such as systemic lupus erythromatus.[76] Future studies may highlight the kinase/s responsible for triggering excess histone proteolysis as potential therapeutic targets (perhaps using small molecules that upregulate or activate these kinases) for certain human disease conditions.

A Role for Histone Tyrosine Phosphorylation in Regulating Chromatin Architecture and Oncogenesis

In addition to the destabilizing role played by the H3 Y99 residue, Singh et al.[14] also found that Y41, the essential tyrosine residue in budding yeast H3, appears to have a small but reproducible role in stabilizing this histone protein. Ectopically expressed mutant H3 carrying the Y41F mutation was degraded at a slightly faster rate than wild type H3, although further investigation revealed that protein stability was not likely to be the essential role of this tyrosine.[14] H3 Y41 makes contacts with the DNA in the nucleosome[38] and is located at the amino terminus of the N α-helix of H3, which may be metastable and unravel under certain conditions, thereby affecting nucleosome remodeling.[77,78] Within a few months of the findings in budding yeast, H3 Y41 was shown to be directly phosphorylated by the protein tyrosine kinase JAK2 (Janus Kinase 2) in human hematopoietic cell lines.[15] Similar to the H2A.X Y142 residue that appeared to serve as the docking site for different factors depending upon its phosphorylation status,[12,51] the phosphorylation status of H3 Y41 appears to be modulating the binding of HP1α (heterochromatin protein 1 α) to histone H3. The binding of HP1α was destabilized by H3 Y41 phosphorylation.[15] Additionally, Dawson et al.[15] showed that inhibition of JAK2 led to reduced expression of leukemia oncogene *LMO2*, probably due to the increased binding of HP1α as a result of reduced H3Y41 phosphorylation. Thus, H3 Y41 phosphorylation may regulate chromatin architecture around certain gene promoters which can have profound implications for hematopoietic cell oncogenesis in humans. Proper (low) JAK2 activity in such cells would maintain low levels of H3 Y41 phosphorylation, thereby allowing HP1α binding and transcriptional repression of target genes such as *LMO2*. Aberrant (high) JAK2 activity in turn would result in high levels of H3 Y41 phosphorylation concomitant with the loss of HP1α binding and an upregulation of target genes such as *LMO2*, which is likely to promote oncogenesis of hematopoietic cells. Interestingly, overexpression of HP1 has been shown to reverse the leukemic phenotype resulting from excessive JAK signaling in fruit flies.[79] Together, these findings may make JAK2 an attractive therapeutic target for certain human hematopoietic malignancies. For future research endeavors along these lines, it would also be helpful to identify the phosphatase/s responsible for reversing the H3 Y41 phosphorylation.

Nuclear JAK2 has not been observed in all cell types and as such it remains to be determined if the H3 Y41 phosphorylation based regulation can be generalized to cell types other than the hematopoietic lineage. However, Dawson et al.[15] suggest that JAK2 is not the only kinase capable of carrying out the phosphorylation of H3 Y41 and other kinases may contribute to a similar mode of regulation in other cell types. Although the Y41 residue in budding yeast H3 is essential,[21,22] it is not known if the same is also true for human cells. It is possible that regulation of chromatin architecture may be the essential role of the H3 Y41 residue in the budding yeast. However, since budding yeast lacks HP1 proteins, it is unclear if a regulation similar to the one described for H3 Y41 phosphorylation in human hematopoietic cells also exists in budding yeast cells. One analogous possibility may be the H3 Y41 dependent regulation of the interaction of the Sir (silence information regulator) proteins, particularly Sir3 and Sir4, which interact with histone H3 and H4 N-terminal tails, to form silent chromatin in budding yeast.[80] However, a specific contribution of H3 Y41 in the binding of Sir proteins has not been evaluated and once again any potential phosphorylation dependent regulation would require the identification of the responsible kinase/s and phosphatase/s.

The Future of Histone Tyrosine Phosphorylation

Among known histone modifications, tyrosine phosphorylation of histones took the longest to be discovered, but it has finally arrived with a bang and there are bound to be numerous exciting discoveries involving this modification in the future. Ongoing studies should be able to explain the finer details of exactly how phosphorylation of H2A.X Y142 regulates the cell's choice to undergo DNA repair or apoptosis following DNA damage. Additionally, these studies may also tell us how we can use this knowledge to influence the cells choice of DNA repair or apoptosis. For example, the efficacy of an important class of anti-cancer drugs (alkylating agents such as cyclophosphamide, crosslinking agents such as cisplatin, topoisomerase inhibitors such as Topotecan), as well as radiotherapy depends on causing DNA damage to cancer cells. Using small molecule inhibitors of EYA phosphatases, it may be possible in the future to selectively modulate the cancer cells to choose apoptosis at lower doses of the DNA damaging agent, thereby limiting the side effects and damage to healthy tissues. At the very least, future studies are likely to provide further insights into the important roles of H2A.X in maintaining genomic stability and cell viability upon DNA damage. Similarly, we are likely to learn more about the essential role of H3 Y41 phosphorylation in yeast and determine if this modification plays a general role in regulating chromatin architecture and function in cell types other than human hematopoietic lines. We will certainly know more about the identity of the different kinases and phosphatases that regulate the reversible phosphorylation of H3 Y41. If warranted by additional studies,

the potential use of small molecule inhibitors of JAK2 (or JAK signaling) to treat certain hematopoietic malignancies may be evaluated within the next few years.

The finding that H3 Y99 and Y41 may be phosphorylated strongly suggests that just about any of the 15 tyrosine residues in the canonical mammalian core histones, including the tyrosines that are inaccessible in the nucleosome due to their involvement in histone-histone or histone-DNA contacts, may be a substrate for phosphorylation in newly synthesized histones, and possibly under certain other conditions. It will be particularly interesting to see if the histone tyrosines that are buried and inaccessible in the nucleosomal context are generally involved in determining the stability of the respective histone proteins. At the very least, we would predict that the major sites of phosphorylation that elicit degradation of excess histones will be distinct from the sites that regulate chromosomal processes. Further, simply based on their accessibility and location within the nucleosome, H3 Y54, H4 Y51 and H2B Y121 would be prime candidates for potential phosphorylation (**Fig. 1**). As in the case of H2A.X Y142 and H3 Y41, phosphorylation at these relatively exposed sites could serve as docking sites for various regulatory factors. Chromatin remodeling[81,82] and transcription induced partial or complete nucleosome disruption[83,84] may allow additional tyrosines to become accessible (H2B Y37, -40, -42 and H4 Y98). Future studies will reveal if phosphorylation of additional tyrosine residues in histones occur and will highlight their physiological roles as well as implications for human health and disease.

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