Published in final edited form as:

Trends Genet. 2013 July ; 29(7): 394–402. doi:10.1016/j.tig.2013.02.003.

WEE1 Tyrosine Kinase, A Novel Epigenetic Modifier

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Abstract

The cell cycle requires cells to duplicate their chromatin, DNA, and histones, while retaining a subset of epigenetic marks, in a highly coordinated manner. The WEE1 kinase was identified as an important regulator during S phase, preventing entry into mitosis until DNA replication has been completed. Interestingly, WEE1 has also emerged as a key player in regulating histone synthesis. It phosphorylates histone H2B at tyrosine 37 in the nucleosomes found upstream of the histone gene cluster, which suppresses histone transcription in late S phase. These observations highlight a dual role for WEE1 as both a mitotic gatekeeper and a surveyor of chromatin synthesis, providing a direct link between epigenetics and cell cycle progression. Importantly, this link has implications for the design of novel epigenetic inhibitors targeting cancers that display elevated expression of this kinase.

Keywords

WEE1; Histones; Tyrosine phosphorylation; Epigenetics; Cancer; Cell cycle

Chromatin integrity and histone synthesis

Chromatin integrity - the synthesis and packaging of the nascent DNA with histones - is critical for proper chromosome condensation, segregation, epigenetic inheritance, and genome stability. Eukaryotic cells tightly regulate synthesis of core chromatin components, during each cell cycle. Although processes that interfere with DNA replication compromise genetic integrity, alterations in histone stoichiometry or mutations in histone genes are linked to chromosome loss, altered chromatin architecture, and cancer [1–6]. In addition, both histones and DNA are modified epigenetically, and inheritance of these epigenetic marks is essential to maintain cell lineage during development [7, 8]. Understanding how cells coordinate the synthesis of chromatin components and retain a subset of epigenetic marks during each cell cycle as well as identifying the key regulatory factors governing these processes are active areas of research.

All eukaryotic cells maintain a precise ratio of core histones to the newly synthesized DNA; both higher and lower ratios of histones to the DNA have deleterious effects (Table 1). The importance of regulating this histone/DNA ratio comes from elegant genetic and

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biochemical studies [1, 2, 9]. In the model eukaryotic organism *Saccharomyces cerevisiae*, histone imbalance created synthetically by selectively over-expressing either the H2A-H2B or H3-H4 dimers, affected mitotic fidelity leading to the loss of chromosomes [1]. Moreover, histone levels are also critical for proper partitioning of chromosomes to the daughter cells [10]. Another undesirable outcome of overproduction of histones, observed in *Schizosaccharomyces pombe*, is a marked increase in chromosomal instability due to the inclusion of canonical H3 along with the centromere-specific H3 variant CENP-A at centromeres, which consequently increases nucleosome density and alters the chromatin structure [10]. Conversely, incorporation of CENP-A (CID in *Drosophila*) at non-centromeric sites leads to the formation of dicentric chromosomes, chromosome breakage during mitosis, and genome instability, which is associated with malignancy [11, 12]. Consequently, cells have developed specific mechanisms to prevent mis-incorporation of CENP-A and maintain chromatin integrity [13]. In addition, transcription of histones is tightly regulated and coordinated with the cell cycle. Genetic studies in yeast revealed presence of a surveillance machinery mediated by the checkpoint kinase, *RAD53*, which monitors histone dosage during replication [14].

Termination of histone gene expression

Just as histone synthesis is exquisitely regulated during the cell cycle, its termination is also precisely synchronized with cell cycle progression [2, 15–17]. Higher eukaryotes have multiple copies of histone genes for the core histones, each encoding a fraction of the total histone protein to deal with the large-scale histone synthesis required for packaging newly synthesized DNA into chromatin during S-phase [18]. These genes are organized in three major clusters in humans, HIST1 (55 histone genes), HIST2 (6 histone genes), and HIST3 (3 histone genes), with copies being arranged in tandem. At the end of S-phase or upon replication inhibition, histone levels are rapidly lowered [2, 19, 20]. In addition, histones are regulated post-transcriptionally in higher eukaryotes through a reduction in mRNA half life at the end of S phase [21–23]. However, the rapid turnover of histone mRNA has to be coupled with cessation of histone mRNA synthesis to achieve the precipitous drop in histone transcript levels observed at the end of DNA synthesis. The process by which higher eukaryotic cells halt histone transcription has remained elusive for a number of years. Recent work demonstrated that transcription of the histone genes is itself regulated by histone modification [17]. Nucleosomes upstream of the mouse histone gene cluster I (*HistI*) were found to be decorated by histone H2B tyrosine phosphorylation at 37 residue (pY37-H2B) precisely at the end of S-phase when DNA synthesis is completed. This mechanism is conserved in *S. cerevisiae*, and mutation of the equivalent site, Y40 to alanine, (*H2BY40A*) leads to a significant increase in all the core histone mRNA levels [17].

A dual role for WEE1 in S phase regulation and histone synthesis

WEE1 is an evolutionarily conserved nuclear tyrosine kinase (Table 2) that is markedly active during the S/G2 phase of the cell cycle [24, 25]. It was first discovered 25 years ago as a cell division cycle (*cdc*) mutant-*wee1*- in the fission yeast, *Schizosaccharomyces pombe* [26]. Fission yeast lacking *WEE1* are characterized by a smaller cell size, and this phenotype has been attributed to the ability of *WEE1* to negatively regulate the activity of cyclin dependent kinase, Cdc2 (Cdc28 in budding yeast and CDK1 in human), in the Cdc2/CyclinB complex [27]. Recently, WEE1 was shown to directly phosphorylate the mammalian core histone H2B at tyrosine 37 in a cell cycle dependent manner. Inhibition of WEE1 kinase activity either by a specific inhibitor (MK-1775) or suppression of its expression by RNA interference abrogated H2B Y37-phosphorylation with a concurrent increase in histone transcription [17]. Interestingly, *S. cerevisiae* lacking the WEE1 homolog, Swe1, also exhibited loss of the equivalent H2B Y40-phosphorylation and histone

gene dysregulation [17]. Collectively, these data suggest a role for WEE1 as a ‘chromatin synthesis sensor’ by two sequential phosphorylation events: (i) Y15-phosphorylation of CDK1 throughout S phase to prevent exit from S phase until DNA replication is completed [17, 28], (ii) Y37-phosphorylation of H2B at the end of S phase to terminate histone synthesis, thus maintaining the right histone-DNA stoichiometry prior to mitotic entry [17].

With more than 50 genes coding for core histones in the *Hist1* cluster in mouse and humans, eukaryotic cells require a strict mechanism to suppress transcription of so many genes. WEE1 deposits pY37-H2B marks at nucleosomes located upstream of *Hist1* cluster to disengage NPAT (Nuclear protein, ataxia-telangiectasia locus) [17], a transcriptional activator of mammalian histone genes and RNA polymerase II [29, 30]. In addition, this epigenetic modification acts as a beacon for the recruitment of a transcriptional repressor, HIRA (histone regulatory homolog A) [17]. HIRA is the mammalian homologue of the yeast HIR proteins and is a component of the histone chaperone complex that spreads across silenced domains to enforce transcriptional repression [31]. HIR proteins bind to the negative regulatory site NEG located at the promoters of seven of the eight yeast histone genes to repress histone transcription [32, 33]. H2B Y37 phosphorylation is enriched in the histone promoters containing the NEG site [17], consistent with a mechanism for HIR recruitment to suppress histone gene transcription.

Cell cycle analysis indicated that cells rapidly exit S-phase once the histone transcription is completed and the pY37-H2B marks are quickly erased [17]. The temporal and transient nature of pY37-H2B suggests that cells may actively recruit a phosphatase to dephosphorylate pY37-H2B before the cells enter mitosis. Furthermore, continuous repression of histone transcription is likely to cripple cells due to a lack of histones to package nascent DNA. Consistent with this, ectopic expression of HIRA which functionally approximates the continuously phosphorylated state of histone H2B, caused arrest in S phase [34]. Although a pY37-H2B-specific phosphatase has not yet been identified, the members of the CDC14 tyrosine phosphatase family are potential candidates. CDC14 is an evolutionarily conserved dual specificity phosphatase [35, 36] that was recently found to interact with Swe1 [37]. Based on the transient nature of H2B Y37-phosphorylation, WEE1 interaction with a tyrosine phosphatase at specific chromatin loci, wherein a kinase recruits the partner phosphatase, seems likely. Other potential candidates include the EYA family of tyrosine phosphatases, which have been shown to dephosphorylate the variant histone H2AX at tyrosine 142 (Box 1).

Box 1

Variant histone tyrosine phosphorylation

Although it has long been known that tyrosine kinases regulate critical cellular processes, it was not discovered until 2009 that they also directly phosphorylate histones [6, 17, 89, 90] (see Table 2). Williams-Beuren syndrome transcription factor (WSTF), a component of the WICH complex (WSTF-ISWI ATP-dependent chromatin-remodeling complex) was the first kinase shown to phosphorylate histone variant H2A.X at tyrosine 142 [90]. This post-translational modification by an atypical tyrosine kinase was shown to play a decisive role when cells were confronted with DNA damage, determining if they initiate DNA repair or undergo apoptosis. The significance of WSTF/pY142-H2A.X signaling became quickly relevant when a protein tyrosine phosphatase, EYA, was found to dephosphorylate pY142-H2A.X in a DNA damage signal-dependent manner [91]. WSTF physically interacts with H2AX and deposits the pY142-H2AX marks specifically in undamaged cells, however, upon DNA damage, formation of a double-strand break (DSB) leads to the recruitment of the meiotic recombination protein-11 (MRE11)–RAD50–Nijmegen breakage syndrome protein-1 (NBS1) (MRN) complex and ataxia-

telangiectasia mutated (ATM) to the DSBs. ATM kinase rapidly phosphorylates the C-terminal tail of H2AX at serine 139 (γ H2AX) [92]. When cells were subjected to ionizing radiation, pY142-H2AX marks were removed by EYA1 and EYA3 phosphatase, promoting a damage repair response by recruitment of MDC1 and associated repair factors [91]. However, failure to remove pY142-H2AX marks in damaged cells leads to the recruitment of pro-apoptotic factors such as JNK1 to H2AX, while inhibiting the recruitment of the damage repair complex, promoting apoptotic response to genotoxic stress.

Epigenetic marks manifested during the cell cycle

In addition to pY37-H2B, a number of other histone marks are regulated in a cell-cycle dependent manner. For example, phosphorylation of serine 10 in the N-terminal tail of histone H3 was shown to be crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis in *Tetrahymena* [38]. Other marks, such as phosphorylation of histone H3 at serine 28 and threonine 11, were also identified to be mitosis-specific histone modifications in mammalian cells [39, 40]. Methylation of histone H3 (K4me3, K9me1, K9me2) and H4 (K20me1) and acetylation of H4 (K5, K16 and K56) are also regulated in cell cycle dependent manner [41]. Although H4K5 acetylation marks are critical for deposition of nascent histones during chromatin assembly by histone chaperones during S phase [42]; they are likewise erased after nucleosome assembly to restore the chromatin structure [41]. The histone deacetylases, HDAC1, HDAC2, or HDAC3, remove H4K5 acetyl marks and their recruitment to newly synthesized DNA is a regulated process [43]. Chicken HIRA has been shown to interact with HDAC1 and 2 [44]. Whether recruitment of HIRA by pY37-H2B establishes a binding platform for the further recruitment of HDACs to deacetylate H4K5ac in the chromatin remains to be established.

In human embryonic stem cells and induced pluripotent stem (iPS) cells the transcriptionally active chromatin marks, H3K4me3 and H3K9ac, were found to be enriched upstream and downstream of the H3 and H4 gene loci but not at the transcription start sites (TSS) in cell cycle- and transcription-dependent manner [8]. Whether pY37-H2B marks occur in pluripotent stem cells and have an active role in cell cycle-dependent transcriptional suppression of histones genes remains to be examined. Because pluripotent stem cells can differentiate into many different cell types, these cells in particular need to tightly regulate chromatin status, which can significantly impact gene expression profiles. Thus, a regulatory mechanism that maintains balanced histone pools during the cell cycle will be critical in stem cells, and given the functional and evolutionary conservation of pY37-H2B marks during cell cycle, a role in regulating histone gene expression in stem cells seems likely.

WEE1 acts as a hub for regulating chromatin integrity

During S-phase, WEE1 appears to mark the completion of not only DNA replication and genetic integrity via Y15-phosphorylation of CDK1 but also histone synthesis by marking chromatin with H2B Y37-phosphorylation before cells enter mitosis [17, 45–47]. Because coupling of DNA synthesis with histone transcription is critical for proper chromatin formation, by regulating both of these processes WEE1 acts as a master regulator of chromatin integrity. Agents that compromise WEE1 function cause mitotic infidelity, chromosome loss, and apoptosis, effect commonly referred to as mitotic catastrophe [48]. Interestingly, in contrast to single-celled eukaryotes such as budding or fission yeast that can survive without *Wee1*, knockout of *Wee1* in mice is embryonic lethal [49]. *Wee1* null embryos do not proceed past the blastocyst stage due to defects in cell proliferation. Moreover, the abnormalities exhibited by *Wee1*-deficient cells are more severe than that noted for other checkpoint proteins such as ATM, which regulates G2/M entry, suggesting

that the loss of proliferation may not be solely attributed to early entry into mitosis [49]. Consistent with this notion, Wee1-deficient cells exhibit increased DNA damage in S-phase as evidenced by increased H2AX S139-phosphorylation, a hallmark of DNA damage, and chromosome aneuploidy [49, 50].

When DNA replication is disrupted by endogenous or exogenous factors such as alkylating agents, oxidative free radicals, ultraviolet-radiation, or nucleotide depletion, cells rapidly mobilize checkpoint and DNA repair proteins to sites of damage. In response to stalled replication forks, the DNA replication checkpoint kinase, ATR (ATM and RAD3-related) is activated. Mammalian ATR regulates activation of the checkpoint kinase CHK1 [51, 52]. CHK1, a serine/threonine kinase not only negatively regulates the CDC25 phosphatase that dephosphorylates CDK1 but also phosphorylates and stabilizes WEE1 [53–55]. Consequently, WEE1 phosphorylates CDK1 in the CDK1/cyclin B complex at tyrosine 15 to prevent mitotic entry until the damaged DNA is repaired (G2/M checkpoint). In addition, WEE1 function appears to be important for the intra-S phase checkpoint as evident from the studies demonstrating that WEE1 inhibitors synergize with CHK1 inhibitors to induce cytotoxicity [56, 57]. During S-phase WEE1 also modulates the activity of CDK1 and CDK2 present at replication origins to control replication initiation (Figure 2). Untimely CDK activity in WEE1 inhibited cells led to a marked increase in the loading of the replication factor, CDC45, at chromatin [58]. As a consequence of augmented replication origin firing, the replication forks slow down and MUS81, a structure-specific endonuclease that directly interacts with WEE1, is recruited to cleave the DNA at the stalled forks generating double strand breaks (DSBs) [58, 59]. It is plausible that WEE1 negatively regulates MUS81 activity at replication forks to prevent untimely DSB formation during DNA replication. Further, the ability of activated WEE1 to phosphorylate Y37-H2B is likely to halt histone mRNA synthesis concomitant with inhibition of DNA synthesis. This is consistent with the earlier observation that showed co-ordinated repression of these two macromolecular processes in response to genotoxic insults [19].

In addition to histone synthesis, chromosome condensation also appears to be regulated by WEE1 kinase [54]. *Drosophila* Wee1 and Grp (the *Drosophila* homolog of CHK1) were found to be crucial for the delay in chromosome condensation when DNA replication was perturbed by treatment with specific inhibitors that interfere with S phase progression or to delay metaphase in cells treated with topoisomerase inhibitors [54]. These observations imply that WEE1 co-ordinates completion of multiple chromosomal processes before cells divide by dynamically surveying events to ensure proper chromatin duplication and segregation (Figure 1). These studies suggest that WEE1 has a critical function in S-phase that is outside its well-established role in regulating mitotic entry. Thus, WEE1 is an important regulator in DNA replication, histone transcription, and chromosome condensation; perturbation of any of these processes profoundly affects chromatin integrity and faithful transmission of genetic and epigenetic information to the progeny (Figure 1).

One significant question is how the WEE1-mediated cytoplasmic signaling cascade (WEE1/CDK1) converges with WEE1-mediated nuclear events (WEE1/H2B) to coordinately regulate cell cycle progression. WEE1 phosphorylates H2B at the end of S phase when the peak phosphorylation of its other substrate, CDK1, is receding [17]. This opens up the possibility that when WEE1 senses completion of DNA synthesis, it switches its substrate preference from CDK1 to H2B. In eukaryotes the origin recognition complex (ORC), Cdc6 protein, and the minichromosome maintenance (MCM) protein complex assemble on chromatin before initiation of DNA replication. CDK1 has been shown to interact specifically with the ORCs in mammalian, *Xenopus*, and yeast cells [60–62]. CDKs regulate DNA replication positively by inducing the initiation of DNA replication at the G₁/S transition and negatively by preventing further rounds of origin firing. WEE1

phosphorylates CDK1 in S phase thus preventing further rounds of origin firing within the same cell cycle [58]. Thus, it is likely that upon completion of DNA synthesis, when WEE1 is prevented from phosphorylating CDK1, it switches to a new substrate, H2B. Thus, by integrating these two seemingly distinct temporal events, WEE1 promotes progression into mitosis with accurately duplicated chromatin.

WEE1-independent modes of histone regulation

Despite its central role in coordinating DNA synthesis and histone transcription, WEE1 is not the only factor regulating these processes. Genetic studies using budding yeast revealed a critical role for the *RAD53* checkpoint kinase in detecting excess histones that are not incorporated into the chromatin. Excess histones bind Rad53, which targets them for degradation by the ubiquitin-proteasome machinery [6, 14]. Furthermore, excess histones in budding yeast sensitized cells to DNA replication inhibitors and lead to loss of chromosomes; conversely, decreasing histone pools by deleting specific histone genes facilitates radioresistance by promoting access of the DNA repair factors to the damaged sites [14, 63, 64]. A recent study identified multiple novel ubiquitin ligases (*Hel1*, *Hel2*, *Pep5* and *Snt2*) in *S. cerevisiae* that ubiquitylate ectopically overexpressed histone H3 and targets it for degradation [65]. Although viable, these E3 ligase mutants displayed differential sensitivity to the replication inhibitor, hydroxy urea (HU). Notably, the *rad53 hel1* double mutants as well as some of the other double mutant combinations did not display increased HU sensitivity compared with the single *rad53* mutant, suggesting that they are either functional redundant or epistatic. Interestingly, increased association of endogenous histones with the histone chaperone Asf1 was observed even in the absence of DNA damage in the *hel1*, *hel2*, *pep5*, and *snt2* deletion strains. These results suggest that defects in histone protein turnover exacerbate cellular sensitivity to genotoxic agents- in part by interfering with the function of histone chaperone/chromatin remodeling enzymes by sequestering them with excess histones.

Other nuclear processes that alter histone stoichiometry can also hamper genome stability [14, 64, 66]. This is evident from several different yeast strains carrying mutations that affect histone levels. For example, excessive core histone mRNA expression has been reported in *S. cerevisiae* lacking *Trf4*, which encodes a poly(A) polymerase, and these mutants are characterized by acute sensitivity to DNA replication inhibitors and defects in chromosome condensation [66]. Although, *trf4* mutants contain wild-type Rad53 that can detect excess histone proteins and target them for degradation by the ubiquitin proteasome machinery, histone mRNA overexpression is detrimental to *trf4* mutants as evidenced by their sensitivity to genotoxic agents and a longer than average S phase. This finding is echoed by the observation that *S. cerevisiae* *H2B Y40A* mutants accumulate excess core histone mRNAs, again suggesting that both transcriptional and post-transcriptional mechanisms are required for maintaining balanced histone pools [17]. Collectively these studies illustrate that eukaryotic cells utilize both genetic and epigenetic controls to tightly regulate histone mRNA levels to ensure genome stability.

WEE1 and cancer epigenetics

The central role of WEE1 in integrating various aspects of cell cycle progression, histone synthesis, and genomic stability makes it an important target for cancer treatment. Gene expression profiling of various tumors revealed that the *Wee1* kinase is overexpressed in Glioblastoma multiforme (GBM) [48, 67], luminal, and triple negative breast cancers (TNBC) [68, 69] as well as malignant melanomas [70]. WEE1 overexpression and the resultant decrease in histone levels could lead to inefficient chromatin packaging, making the DNA more accessible to the DNA damage repair machinery and promoting

radioresistance [64]. The ability of WEE1 to downregulate histone levels could explain why cancer cells become dependent on its epigenetic activity. In addition to acquiring radioresistance, decreased nucleosomal packaging and consequently local alterations in chromatin architecture may activate transcription of pro-proliferative genes or even oncogenes that are otherwise kept in check in normal cells.

Overexpression of WEE1 in certain cancer types could be exploited by using WEE1-specific small molecule inhibitors such as MK-1775 that is in clinical trials [71]. Such inhibition of WEE1 in actively replicating cancer cells would increase histone dosage and, when combined with DNA damaging agents, would interfere with the DNA repair machinery and compromise genome integrity. Consistent with this hypothesis, the WEE1 inhibitor MK-1775 was able to radiosensitize human lung, breast, skin, brain, and prostate cancer cells to DNA damaging agents, and WEE1 inhibitors were shown synergize with CHK1 inhibitors to induce cytotoxicity [56, 72–76]. Moreover, breast cancer cells treated with hydroxyl urea (which inhibits DNA synthesis) and WEE1 inhibitors were found to contain a marked increase in disorganized mitotic spindles and abnormal mitoses [56].

Enzymes that promote or reverse histone modifications have emerged as major targets for the development of small molecule inhibitors, commonly referred as epigenetic inhibitors. Many of these are already in different stages of clinical trials with significant success in hematologic cancers [77]. The precision with which histone modifying enzymes modify a specific amino acid residue in a histone makes them ideal candidates for drug discovery efforts, however, because of a highly heterogeneous nature of disease, the efficacy of the epigenetic inhibitor is likely to be seen in only a subset of cancer patients that are addicted to the activity of the histone modifying enzyme. One well studied example is histone methyltransferase EZH2 that deposits the repressive mark H3K27me3. Two somatic heterozygous mutations in EZH2, Y641 and A677, have been identified in about 22% of diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma, which caused increased H3K27me3 levels [78–82]. Recently, two small-molecule inhibitors of EZH2 were identified, EPZ005687 and GSK126, which suppressed global H3K27me3 levels and reactivated silenced EZH2 target genes leading to inhibition of EZH2 mutant DLBCL cell line proliferation and the growth of xenograft tumors [83, 84]. This and other early successes, suggest that WEE1 inhibition may be a useful strategy for treatment of a subset of cancers.

Cancer is a complex disease, differences in biology and outcomes exist not only among various clinical states but also within each patient. Personalized medicine offers the potential to optimize treatment for a given patient, based on molecular biomarkers that drive individual variability or drug responses. Thus, elucidation of abundant H2B Y37-phosphorylation in a subset of GBM, TNBC or malignant melanoma tumor biopsies could be a ‘companion diagnostic’ test for administration of WEE1 inhibitor such as MK-1775. This ‘personalized therapy’ for a subset of WEE1-positive GBM, TNBC or malignant melanoma patients could be a significant development as limited therapeutic options and no targeted therapeutic modalities are currently available for these cancers with low survival rates.

Defects in chromatin architecture were recently discovered to play a critical role in pediatric and young adult GBM pathogenesis, a lethal brain tumor in both adults and children [3–5, 85]. Exon sequencing revealed two somatic mutations, K27M and G34R/G34V in the histone H3 variants, H3.3 and H3.1. Interestingly, mutations in ATRX (α -thalassaemia/mental retardation syndrome X-linked) and DAXX (death-domain associated protein) were identified in every tumor harboring a G34R/G34V mutation. ATRX and DAXX are subunits of a chromatin remodeling complex that is essential for H3.3 incorporation at pericentric

heterochromatin and telomeres. It has been suggested that the alterations in heterochromatinization is the major consequence of the mutations in H3.1, H3.3, and the ATRX-DAXX chromatin remodeling pathway, leading to alternative lengthening of telomeres and expression of specific gene profiles [3–5]. Whether, GBM exhibits nucleosomal packaging defects selectively in children by acquiring histone mutations, and in adults by WEE1 overexpression would require more studies. However, the alteration in heterochromatic states appears to be the major mechanism of pathogenesis in GBM.

Concluding remarks

The discovery of H2B Y37-phosphorylation by WEE1 provides a direct link between the cell cycle kinase and an epigenetic mark that helps to maintain chromatin homeostasis. This finding raises an important question of whether WEE1 utilizes its ability to phosphorylate H2B to accomplish its other well known function as a mitotic gatekeeper. This expands our perspective of how we define the role of WEE1 in cell cycle regulation and its role in various malignancies, especially those which over express WEE1 kinase; these cancer cells could utilize alterations in histone levels to confer selective proliferative advantage and radioresistance.

Identification of WEE1 epigenetic activity raises several important questions in normal physiology as well as in disease pathology. Are there auxiliary loci epigenetically marked by pY37-H2B modification in cancers with aberrant WEE1 expression? If so, do cancer cells employ a different set of epigenetic readers to read H2B Y37-phosphorylation and are there common motifs that distinguish the promoters marked with this specific modification? It is still unclear at this point if pY37-H2B modulates chromatin architecture by recruiting chromatin-remodeling proteins and thereby impacting patterns of local gene expression. Another area to explore is whether this histone Tyr-modification potentially cross talks with other histone modifications such as the H2B ubiquitylation and H4K5 and H3K56 acetylation, which have known roles in chromatin replication or repair [8, 41, 86–88]. Further research aimed at answering these questions is critical for a clear understanding of the global regulatory role of histone Tyr-phosphorylation and the development of WEE1 inhibitors as potential cancer therapeutics.

Acknowledgments

We apologize to those authors whose work could not be cited owing to the space constraint of reference citations. We thank Dr Akash Gunjan for critical reading of manuscript, Dr. Jozef Sychala, VisiScience for help with Figure 1. We declare competing financial interests; we are named as inventors on US patent application 61/583,864 and international application PCT/US2013/020395 titled “Antibodies specific for phosphorylated histones and uses thereof.” This work was supported in part by Department of Defense (W81XWH-12-1-0248) to K.M. and by the National Cancer Institute, NIH (1R01CA135328) and Moffitt Lung Cancer Spore to N.P.M.

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Box 2**Core histone tyrosine phosphorylation**

The first core histone shown to be Tyr-phosphorylated in mammalian cells was histone H3. A non-receptor tyrosine kinase, Janus kinase 2 (JAK2) directly phosphorylates H3 at tyrosine 41, preventing binding of heterochromatin protein 1 α (HP1 α) [89]. HP1 α represses the transcription of heterochromatic genes and preserves centromeric architecture [93, 94]. JAK2 is frequently hyperactivated in hematological malignancies, leading to the upregulation of *LMO2*, a gene important for tumor angiogenesis [89]. In addition, pY41-H3 marks other key lineage-specific hematopoietic genes [95]. Treatment with JAK2 inhibitors abrogates pY41-H3 marks, reduces levels of the pluripotency regulator Nanog, and increases recruitment of HP1 α levels at the Nanog promoter [96]. In addition to Y41, H3 was also shown to be phosphorylated at Y99 in *S. cerevisiae* [6]. Interestingly, it was the non-chromatin bound histones, which were phosphorylated in a Rad53-dependent manner leading to their polyubiquitylation and degradation by proteosomal machinery [6]. The ubiquitin conjugating enzymes (E2) Ubc4 and Ubc5 and the ubiquitin ligase (E3) Tom1 (temperature dependent organization in mitotic nucleus 1) were shown to be involved in the polyubiquitylation of excess histones [6]. Taken together with histone transcriptional suppression role of H2B Tyr37-phosphorylation [17], these data indicate that cells not only shut down histone transcription upon completion of DNA synthesis or when exposed to DNA damaging agents, but they also degrade excess histones that are not incorporated into chromatin to prevent harmful effects of non -chromatin bound histones.

It is likely that there are other modes of histone Tyr-phosphorylation, especially in mammalian cells where many non-receptor tyrosine kinases could potentially target 15 distinct tyrosine residues in the core histone- (H2A- Y39, Y50, and Y57; H2B- Y37, Y40, Y42, Y83, and Y121; H3- Y41, Y54, and Y99; H4- Y51, Y72, Y88, and Y98), and 2 in the linker histone H1 (Y88 and Y119). The transient nature of histone Tyr-phosphorylation may be the reason why only a few examples these epigenetic changes have been identified to date. However, these tyrosine residues are highly conserved (Table 2), suggesting that new examples found in yeast or other tractable genetic systems are likely to be relevant in mammalian cells as well.

Highlights

- WEE1 phosphorylates histone H2B at tyrosine 37 in the late S phase
- H2B Tyr37-phosphorylation downregulates expression of multiple histone genes
- WEE1 kinase has emerged as a novel epigenetic writer
- pY37-H2B epigenetic marks decorate several DNA and histone modifying genes

Figure 1. Schematic outlining WEE1 functions

WEE1 is emerging as a critical surveyor of chromatin integrity across the evolutionary landscape by regulating histone synthesis and the cell cycle. It controls genome stability by regulating the activity of factors such as CDKs involved in replication initiation during S phase. During DNA replication, it negatively regulates the activity of the Mus81 endonuclease at stalled replication forks to prevent formation of DNA double strand breaks and preserve genetic integrity. Importantly, it appears to couple DNA synthesis with histone synthesis due to its ability to prevent mitotic entry until DNA synthesis is completed and to switch off histone transcription, by modifying core histone H2B, at the end of S phase. These functions are likely to be critical for its ability to delay chromosome condensation and prevent aneuploidy.

Figure 2. A model for WEE1 in chromatin integrity during replication stress

Replication protein A (RPA) (small magenta circles) coats ssDNA formed during DNA replication and DNA repair which facilitates the localization of Ataxia-Telangiectasia mutated and RAD3-related (ATR) kinase to sites of DNA damage in both human and yeast systems. Activated ATR phosphorylates the downstream checkpoint kinase-1 (CHK1) which in turn phosphorylates and activates WEE1. In addition to WEE1 activation, CHK1 also phosphorylates and inactivates CDC25, a phosphatase that dephosphorylates CDK1. WEE1 plays a crucial role in maintaining chromatin integrity during DNA damage or replication stress by performing two distinct phosphorylations; first, it phosphorylates CDK1 at Y15 preventing entry into mitosis so as to allow repair of damaged DNA. Second, it actively downregulates histone gene transcription by marking H2B at Tyr37 in nucleosomes upstream of histone cluster 1 (Hist1). H2B Tyr37 phosphorylation prevents binding of the transcriptional coactivator NPAT and RNA polymerase II and recruits the histone chaperone HIRA upstream of the *Hist1* cluster thereby avoiding overproduction of histones. The collective outcome of these events is initiation of intra-S phase and G2 checkpoints in response to DNA damage or replication stress and maintenance of chromatin integrity during repair of damaged DNA, prior to entry into mitosis.

Table 1

Known phenotypes associated with histone alterations

Histone	Species	Molecular Phenotypes	Refs
Alteration of histone stoichiometry	<i>S. cerevisiae</i>	Chromosome loss, DNA damage sensitivity, and slow growth	[9, 97]
Repression of H2B RNA synthesis in yeast	<i>S. cerevisiae</i>	Cell cycle arrest	[98]
Change in dosage of H2B	<i>S. cerevisiae</i>	Altered gene expression	[99]
Histone H1 ^o variant overexpression	Mouse	Reduction in steady state transcription of all RNA pol II regulated genes and transient inhibition of G1 and S phase progression; increase in nucleosome spacing	[100, 101]
Histone H1 ^c variant overexpression	Mouse	Marked increase in gene expression at some loci	[100]
Synthesis of histones in non-replicating cells	<i>Drosophila</i>	Developmental arrest and zygotic lethality	[102]
Embryos lacking the three H1 subtypes (H1c, H1d, and H1e),	<i>Drosophila</i>	Die by mid-gestation with a broad range of defects	[103]
Partial depletion of Histone H4	<i>S. cerevisiae</i>	Accumulation of recombinogenic DNA lesions	[104]
Overproduction of histones	<i>S. pombe</i>	Incorporation of canonical H3 along with H3 variant CENP-A at centromeres	[10]
Somatic mutation in H3F3A gene, K27M and G34R/G34V, encoding histone H3.3	Human	Mutations unique to pediatric and young adult glioblastomas, associated with lengthening of telomeres and specific gene expression profiles	[3–5]
Somatic mutation in HIST1H3B, K27M and G34R, encoding histone H3.1	Human	Mutations unique to pediatric glioblastomas	[4]
D123N mutation in H3F3a	Zebrafish	Eliminates the cranial neural crest-derived head skeleton	[105]

Table 2

Histone Tyrosine Kinases

Kinase	Histone	Tyrosine residue	Interactors	Function	Lethality in budding yeast	Human disease association	Refs
WSTF	H2AX	142	APBB1, PTB-domain containing adaptor protein Fe65	Maintains DNA damage induced foci formation	Site is absent in yeast	Williams syndrome	[90]
WEE1	H2B	37	Recruitment of HIRA	Transcriptional Suppression of histone genes	Not lethal in normal growth conditions	Glioblastoma, melanomas, TNBC	[17, 48, 67, 68, 70, 106]
JAK2	H3	41	Prevents recruitment of HP1	Transcriptional activation	Lethal	Leukemia	[89]
* RAD53 (homolog of CHK2)	* H3	*99	Recruitment of ubiquitin conjugating enzymes Ubc4-Ubc5	Degradation of excess histones	Not lethal in normal growth conditions	Not known	[6]

* identified only in *S. cerevisiae*