Flickin’ the ubiquitin switch
The role of H2B ubiquitylation in development

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Key words: H2B, histone, ubiquitylation, development, deubiquitylation, Drosophila melanogaster, Arabidopsis thaliana, carcinogenesis

Abbreviations: H2Bub1, monoubiquitylated H2B; me2, dimethylated; me3, trimethylated; DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; SAGA, Spt-Ada-Gcn5 acetyltransferase; GMPS, guanosine-5'-monophosphate synthetase; MADS, Mcm1, agamous, deficiens, Srf; FACT, facilitates chromatin transcription; FLC, flowering locus C; DSB, double-stranded break; MOF, males absent of the first; CDK, cyclin-dependent kinase; modENCODE, model organism encyclopedia of DNA elements

The reversible ubiquitylation of histone H2B has long been implicated in transcriptional activation and gene silencing. However, many questions regarding its regulation and effects on chromatin structure remain unanswered. In addition, while several studies have uncovered an involvement of this modification in the control of certain developmental processes, a more general understanding of its requirement is lacking. Herein, we present a broad overview of the pathways known to be regulated by H2B ubiquitylation, while drawing parallels between findings in disparate organisms, in order to facilitate continued delineation of its spatiotemporal role in development. Finally, we integrate the findings of recent studies into how H2B ubiquitylation affects chromatin, and cast an eye over emerging areas for future research.

Introduction

The formation of a multi-cellular organism requires co-ordinated rounds of cellular proliferation, differentiation and apoptosis, to generate, specify and shape body tissues.1,4 Strict regulation of proliferation and apoptosis are important for preventing uncontrolled cell growth, the hallmark of cancer.4 Meanwhile, spatial and temporal control of gene expression gives rise to a myriad of specialized cells from a single genome.5 Such differential expression patterns are initially established through transient signals, but long-lasting, epigenetic changes are required to maintain these patterns throughout the cell lineage.6 Amongst these changes is the reversible addition of a single ubiquitin moiety to a specific lysine residue of histone H2B.7 Ubiquitylated H2B (H2Bub1) is a requirement for di- and tri-methylation of lysines 4 and 79 of histone H3 (H3K4me2/3 and H3K79me3), but its biological effects extend beyond downstream regulation of these modifications.8,9 Ubiquitylation and subsequent deubiquitylation of H2B are both required for transcriptional elongation of certain stress-inducible genes, and removal of the ubiquitin tag from H2B enables the establishment of telomeric silencing, through the association of Sir factors with chromatin.10,11

Certain roles of H2Bub1 remain controversial; recent studies have discredited the purported role of H3K79me3 in silencing, although the requirement for H2B deubiquitylation has not been investigated further.12,13 However, the purpose of this review is not to describe how H2Bub1 may regulate transcription, telomeric silencing and trans-histone modifications (reviewed in ref. 14 and 15). Instead, we intend to give an overview of the developmental processes affected by H2Bub1. As research into the mechanisms by which ubiquitylation of H2B affects processes on the DNA template continues, there is also an increasing trend towards characterizing the developmental processes that are affected by this histone modification in multi-cellular organisms. This is no trivial task; the identification of direct targets of H2B ubiquitylation is complicated by pleiotropic phenotypes.16 Further difficulties arise from the reiterative nature of histone genes in higher eukaryotes; whereas H2B is encoded by only two genes in budding yeast, there are at least seventeen human genes encoding H2B and its variants, spread throughout the genome.17,18 Such redundancy can be countered to an extent; transfection of a ubiquitylation-deficient mutant H2B construct into human cell lines causes a dominant negative reduction of endogenous H2Bub1, and it is now possible to replace the entire complement of histone genes with mutant variants in the fruit fly Drosophila melanogaster.19,20 Studying the enzymes that target H2B for de/ubiquitylation is also associated with issues of mutant viability, multiple targets and redundancy, all of which need to be considered when investigating the effects of H2B ubiquitylation on development.21,23 Bearing in mind these limitations, we shall now summarize the current understanding of said requirement, and hope that this will expedite future studies into developmental control by H2Bub1.
H2Bub1 and Signal Transduction: Evidence from Flies

Notch signaling. Several signaling pathways are known to regulate histone modifications and other epigenetic changes. One such pathway, Notch, is involved in specifying divergent cell fate, and therefore plays a key role in the generation of multicellular organisms (Fig. 1). In the fruit fly, H2B is ubiquitylated by dBre1, an E3 ubiquitin ligase. Mutant clones of dBre1 exhibit defects characteristic of disrupted Notch signaling, such as notches in the wing margin, alluding to a possible role for mutant cells, and transfection ofates expression of a Notch-specific reporter gene. As expected, late histone modifications and other epigenetic changes. One signaling pathways are known to regulate Notch signaling.

Expression of certain Notch target genes was also found to be reduced or lost in dBre1 mutant cells, and transfection of dBre1 into fly S2 cells stimulates expression of a Notch-specific reporter gene. As expected, the developmental defects of a Notch mutant background were enhanced by introduction of a dominant negative dBre1 variant, but surprisingly, overexpression of wild-type dBre1 had the same effect. The reasons for this remain elusive, but suggest that tight control of dBre1 activity is perhaps required for appropriate target gene activation.

H2Bub1 and downstream H3K4me3 are dependent on Rtf1, a component of the Paf1 complex in budding yeast. Knockdown of the fruit fly homologue, dRtf1, in vivo also dramatically reduces H3K4 trimethylation, and enhances the severity of cleavage of the Notch receptor. Drug treatment is able to partially restore the number of intestinal stem cells, suggesting that excess H2Bub1 results in inappropriate activation of Notch target genes. Buszczak et al. (2009) propose that the high levels of Scny that they observe in stem cells maintain low levels of H2Bub1 at Notch target genes and other genes required for differentiation, preventing their activation (Fig. 1). Scny also appears to have a role in apoptosis, which will be discussed in a later section.

Wingless signaling. Recent evidence suggests that H2Bub1 may interact with a second signaling pathway, Wnt, through downstream regulation of H3K79me3. Mohan et al. (2010) discovered that β-catenin physically interacts with human Dot1L, a H3K79 methyltransferase. β-catenin is a component of the Wnt signaling pathway (Wingless in flies), which, like Notch, is a major signal transduction cascade in metazoans, and is integral for stem cell renewal and proliferation (Fig. 2). Pursuing the implication that H3K79 methylation may play a regulatory role in Wingless signaling, the authors generated flies with a targeted knockdown of grappa, a Dot1L orthologue, within the posterior compartment of the wing imaginal discs. This was found to reduce levels of high-threshold Wingless targets, but not low-threshold genes. A similar reduction of high-threshold genes was also observed on knockdown of dBre1, suggesting that

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**Figure 1.** In *Drosophila melanogaster*, the transmembrane receptor Notch binds to the ligand Delta or Serrate, resulting in cleavage of Notch, and translocation of the Notch intracellular domain (NICD) to the nucleus. NICD functions as a transcription factor that drives the activation of target genes, through an interaction with gene-bound Su(H) that results in the eviction of co-repressors (CoR) and recruitment of co-activators (CoA). The E3 ligase dBre1 is required for full-expression of Notch target genes, through an unknown mechanism. Rtf1 is also required for Notch signaling and H3K4 methylation, which may be mediated through H2B ubiquitylation by dBre1. The histone chaperones ASF1 and NAP1 are required for Notch gene repression, and interact with the H3K4 demethylase LID/KDM5 and Su(H). Loss of the H2B ubiquitin protease Scrawny (Scny) induces stem cell differentiation, and this can be prevented by inhibiting Notch signaling, suggesting that Scny represses this pathway prior to differentiation.
the requirement of H2Bub1 for H3K79me3 regulates short-range Wingless signalling.5,8

The reduction in the expression of both Notch and Wingless target genes in dBre1 mutants is particularly interesting when considered against the well-established interaction between the two signaling pathways.5,21,32 Indeed, dBre1 was first identified as the result of a mutation that modified the wing notching phenotype caused by depletion of the fly β-catenin homologue.22 As such, it seems possible that H2Bub1 serves to integrate these two cascades at the level of transcriptional activation. Regulation of H2Bub1 is seemingly integral to an undeniably complex concert of signaling events, which coordinates stem cell regulation.

**Ecdysone signaling.** Further to the requirement for Scny, a second H2B ubiquitin protease, Nonstop, also plays a role in fruit fly development.33-35 First identified as the result of a screen for mutations that affect neuronal connectivity in the brain, Nonstop expression in glia was subsequently found to be required for the migration of these cells into the axonal projection field.34,35 Nonstop is the fly orthologue of yeast Ubp8, a component of the SAGA complex required for the activation of certain stress-inducible genes (Table 1).33,35 Weake et al. (2008) demonstrated that Nonstop may affect glial migration as part of the SAGA complex, for mutations that affect neuronal connectivity in the brain,33-35 of considerable interest is the finding that reducing the deubiquitylation activity of SAGA in muscle results in a preferential downregulation of genes required specifically for muscle development.36 As such, it seems that appropriate glial migration and tissue-specific development may depend on gene activation through SAGA. Weake et al. (2008) note that mutations of nonstop and other SAGA components also result in decreased expression of several genes that are regulated by ecdysone, a steroid hormone that regulates molting and metamorphosis in arthropods (Fig. 3).35,37

More direct evidence for an interaction between H2B deubiquitylation and ecdysone signaling comes from studies into the action of the ubiquitin protease USP7 (Table 1). This enzyme was found to co-purify with guanosine-5’-monophosphate synthetase (GMPS) in extracts from fruit fly embryo, and GMPS is required for deubiquitylation of H2B by USP7 in vitro.38 Both proteins co-localize to ecdysone-response gene loci, and the developmental expression pattern of these genes is disrupted in USP7 and GMPS mutants.38 USP7 is lost from these gene loci following the ecdysone pulse prior to pupariation, and this coincides with an increase in the levels of dBre1 and RNA Polymerase II at these genes, strongly suggesting that regulation of H2B ubiquitylation helps mediate ecdysone-response gene activation (Fig. 3).39

USP7 and GMPS were also shown to localize to the Antennapedia and Bithorax homeotic gene clusters, and mutation of the encoding genes enhanced homeotic transformations observed in Polycomb mutants.38 These findings strongly suggest an involvement of USP7/GMPS in the silencing of homeotic genes, and thus body plan establishment, possibly through deubiquitylation of H2B.38 This becomes a particularly tempting hypothesis when we consider that homeotic gene expression is altered upon mutation of the dBre1 orthologue in *Arabidopsis thaliana*, and that knock down of RNF20, the human orthologue, results in diminished transcription of Hox genes (Fig. 5).36,40

Finally, the involvement of at least three H2B ubiquitin proteases (Scny, Nonstop and USP7) in fly signaling is certainly worthy of further investigation in future. While it is apparent that enzyme-specific substrates contribute to the lack of redundancy, might differential spatiotemporal control of H2B deubiquitylation also be important?30,38 We can perhaps look to yeast for insight. Ubp8 and Ubp10 are the yeast homologues of Nonstop and Scny respectively (Table 1).35,38 Global levels of H2Bub1 are increased to a greater extent in mutants deficient for both enzymes than either one alone, indicating non-overlapping targets of these proteases.11,23 This is reflected in their distinct functional roles, and patterns of localization; Ubp10 is preferentially localized to silenced regions of the genome, while Ubp8 shows no such preference.11,23 The silencing factor Sir4 is required for telomeric localization of Ubp10 through an unknown mechanism, and Ubp8 is recruited to stress-inducible genes as part of

<table>
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<tr>
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<td>USP7 [HAUSP] (oncogene)</td>
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<td>NA</td>
<td>Ubp26 (prevention of early flowering; vegetative growth; gene repression)</td>
<td>NA</td>
<td>USP48 [USP31] (regulation of NFkappaB activation)</td>
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Homologous proteins are displayed in the same row. Alternative names are indicated in square brackets. Reported functions or oncogenic potential are indicated in parentheses. NA indicates that a homologous protein has not been identified in the relevant organism.
Ubiquitylation and vernalization. Ubiquitylation and deubiquitylation of H2B are important at multiple stages of development in Arabidopsis.\textsuperscript{16,41-47} Mutations in the plant orthologues of the yeast H2B ubiquitin protease \( \text{ubp8} \) and \( \text{ubp26} \) affect the vernalization response, flowering, and plant size, as well as other pleiotropic phenotypes.\textsuperscript{16,42-46} Four independent studies later found that mutant alleles of \( \text{hub1} \) and \( \text{hub2} \) give rise to an early-flowering phenotype.\textsuperscript{43,45} The early flowering phenotype of the \( \text{hub2} \) mutant is similar to that observed in mutants of the PAF1 transcriptional elongation complex,\textsuperscript{16,42-46} suggesting that the absolute level of this protein is important for development.\textsuperscript{41} The defect in early flowering is associated with a decrease in H3K36me3 within the FLC gene (Fig. 4).\textsuperscript{41} The FACT (FAcilitates Chromatin Transcription) complex is a transcription elongation factor that interacts synergistically with H2Bub1 in yeast and humans.\textsuperscript{51}

**Figure 2.** \( \beta \)-catenin binds to a complex (consisting of axin, adenomatous polyposis coli (APC) and two kinases, casein kinase (CKI) and glycogen synthase kinase 3\( \beta \) (GSK3\( \beta \)), which catalyses phosphorylation of the former, that in turn results in its polyubiquitylation and degradation. Binding of Wnt to its receptors, Frizzled and LRP, triggers the recruitment of the complex to the cell membrane, thereby causing the degradation of axin and the release of \( \beta \)-catenin. Free \( \beta \)-catenin is able to enter the nucleus, where it binds to transcriptional regulators of the TCF (T cell factor) family, and activates TCF-bound genes. \( \text{dBre1} \) and \( \text{grappa} \) (Dot1) are required for full activation of high-threshold (i.e., short-range) Wingless target genes, possibly through H2B ubiquitylation and downstream H3K79 trimethylolation.

Unexpectedly, the results of double mutant analysis suggest that HUB1 and SSRP1/SPT16 have independent effects on flowering, despite both regulating levels of the FLC gene, which encodes a MADS-box repressor.\textsuperscript{46} FACT and H2B ubiquitylation do, however, appear to affect certain phenotypes (leaf shape and vernalization, silique size) through the same pathway (Fig. 4).\textsuperscript{46}

**Requirement for deubiquitylation.** Consistent with a reported role for both H2B ubiquitylation and deubiquitylation in transcriptional elongation, a recent study has demonstrated a requirement for the Arabidopsis H2B ubiquitin protease UBP26 (homologous to human USP31; Table 1) in driving expression of FLC.\textsuperscript{10,41,52} As for the \( \text{hub1} \) mutations, \( \text{ubp26} \) mutations also result in early-flowering and smaller leaves.\textsuperscript{41} In addition, overexpression of UBP26 also results in decreased plant size, suggesting that the absolute level of this protein is important for development.\textsuperscript{41} The defect in early flowering is associated with a decrease in H3K36me3 within the FLC gene (Fig. 4).\textsuperscript{41} This is in agreement with the requirement of H2B deubiquitylation for tri-methylation of H3K36 at certain genes in yeast.\textsuperscript{10} Strangely, H3K36me2 levels are unaffected, despite the increase observed in mutants of \( \text{hub1} \) and the decrease in mutants of the yeast H2B ubiquitin protease \( \text{uph8} \), indicative of distinct mechanisms of transcriptional control between yeast and higher eukaryotes.\textsuperscript{16,43,45}

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A second post-translational modification on histone H3, tri-methylation of K27, is also increased at the transcription on histone H3, tri-methylated K27.5 Luo et al. (2008) thus suggested that deubiquitylation of H2B by UBP26 may be a prerequisite for such H3K27 tri-methylation, in agreement with the finding that RNAi depletion of the H2B ubiquitin protease USP7 in fly S2 cell culture results in a global decrease of H3K27me3.38,47 The disparate effects of UBP26 in two different genetic backgrounds (being required for either FLC transcription or PHE1 repression) hint at complex, gene differential effects and emphasize the redundancy of H2B ubiquitin proteases in higher eukaryotes, highlighting a need to confirm findings in reduced systems.41,47

**H2Bub1 In Gametogenesis**

**Meiosis.** Meiosis is the cornerstone of sexual reproduction, as it generates haploid cells (gametes or spores) required for the formation of a diploid organism genetically distinct from both parents.55 Substantial chromatin changes, including modifications of histones, are observed prior to and during meiosis, as well as during the maturation of gametes (gametogenesis).55

A yeast strain specifically impaired in ubiquitylation of H2B (through mutation of the target lysine to arginine, K123R) was found to be unable to form spores on account of meiotic prophase arrest.7 Detailed characterization of the H2B K123R mutant in a rapidly sporulating genetic background revealed that the formation of double-stranded breaks (DSBs) during meiotic pachytene is delayed, and the frequencies of DSBs at certain loci were reduced.56 Furthermore, the levels of ectopically-induced DSBs are unaffected by H2B K123R, suggesting that ubiquitylation is required for recruitment of a DSB-creating complex at specific loci during meiosis, rather than having a general effect on DSB formation.56

In an intriguing parallel, two recent papers reported that H2Bub1 is required for the repair of DSBs in human cells.9,47 RNF20 is recruited to DSBs, presumably underlying the observed increase of H2Bub1 upon DNA damage.19,57 Preventing H2B ubiquitylation impedes the accumulation of repair proteins, with the histone mark acting upstream of the chromatin remodeling factor SNF2h.99

**Spermatogenesis.** The mammalian enzymes HR6A (RAD6A) and HR6B (RAD6B) are the major E2 conjugating enzymes for H2B ubiquitylation in their respective species.58 Paralleling the sporulation defects observed in yeast, male mice deficient for HR6B are infertile, due to abnormal spermatogenesis.99 It remains unclear as to whether there is a requirement for HR6B-mediated ubiquitylation of H2B in this process, as Baarends et al. (1999) detected H2Aub1, but not H2Bub1 during meiotic pachytenic and in elongating spermatids.60,61 An earlier study was able to detect H2Bub1 in elongating rat spermatids however, through the use of less stringent precipitation.62 Downregulation of several autosomal genes was observed in the HR6B knockout, including many genes that regulate the cell cycle and embryonic development.63 Decreased silencing at centromeric regions of spermatocytes is also observed, which, akin to telomeric silencing in yeast, may involve altered Sir protein distribution in the absence of H2Bub1.64 Subsequent studies revealed that the mutant also exhibits defects during pachytene, with alterations in chromatin structure and increased meiotic recombination, while female meiosis was unaffected.64 Conversely, HR6A is not required for spermatogenesis, but HR6A null females are sterile, due to early arrest of embryo development.65 It is speculated that the contrasting phenotypes observed in HR6A and HR6B-deficient animals
Regulation of H2Bub1 during the Cell Cycle and Apoptosis

Cell cycle progression. Progression of the cell cycle has dual significance for histone modifications; on the one hand, some modifications have roles in regulating chromatin structure during DNA replication and mitosis, whereas on the other, the global epigenetic pattern needs to be inherited by the ensuing daughter cells to ensure appropriate cell fate. In yeast, the H2B K123R mutation causes an increase in doubling time and the proportion of large budded cells, indicative of mitotic defects. This mutation was subsequently found to contribute to mitotic exit, through promoting release of the phosphatase Cdc14 from nucleolar chromatin.

Although less is known about the relationship between H2Bub1 and cell cycle regulation in multi-cellular organisms, studies on various species allude to some involvement. Mutations of hub1 and hub2 in Arabidopsis cause a decrease in the growth rate of leaves and roots, due to a block in the G2-M transition and the resulting increase in cell cycle duration. This mitotic block also contributes to early exit from the cell cycle and entry into endoduplication in some cells. Several genes involved in mitotic transition and cytokinesis are downregulated in hub1 mutant plants, including 66 of 82 genes that normally exhibit peak expression in mitosis. A study into the human Rad6 orthologue, hHR6A, suggests that regulation of cell cycle progression by H2Bub1 may be conserved in mammals. The major regulators of cell cycle progression are cyclin-dependent kinases (CDKs) and their regulatory cyclins, which phosphorylate numerous substrates required for entry into and progression through the various stages. Several cyclin-CDK complexes are able to phosphorylate hHR6A in vitro, and phosphorylation of hHR6A at serine 120 in vivo is abolished through the use of an inhibitor of CDK-1 and 2. Phosphorylation of this residue was found to increase ubiquitylation of H2A in vitro, and whereas wild-type hHR6A is able to rescue the proliferation defect of yeast rad6Δ strains grown at 37°C, a non-phosphorylatable hHR6A mutant cannot compensate for loss of Rad6. It was also reported that phosphorylation of hHR6A is greatest at G2/M phase, and this coincides with high levels of H2Bub1, which are reduced thereafter. Redundancy of action of HR6A and HR6B may explain the viability of deletion mutants of HR6A and HR6B in mouse, supposing that cell cycle progression requires H2B ubiquitylation mediated through either protein.

Apoptosis. While this review has thus far concentrated on cellular proliferation and differentiation, just as important may result from dose-dependent effects in the respective gametes, or from divergent interactions with putative E3 ligases. Again, the role of H2B ubiquitylation, if any, remains unclear; levels of H3K4me3 remain unaltered in the HR6A deficient oocytes, but the effects on H2Bub1 in these cells is as yet unaddressed.

Finally, male mice deficient for the E3 ligase RNF8 are sterile, and this correlates with a decrease in both H2Aub1 and H2Bub1 in elongating spermatids. The sterility appears to result from a defect in the replacement of histones with transition proteins, disrupting DNA compaction in late spermatids. H2A and H2B ubiquitylation may be required for recruitment of the acetyltransferase MOF (males absent of the first) to chromatin, which targets H4K16 for acetylation. Such acetylation may destabilize nucleosomes, thereby facilitating histone removal and enabling spermatid compaction to occur.
splice isoforms (Table 1). The mechanisms are unclear, but the authors of this study postulate that the longer Scny isoform prevents apoptosis by deubiquitylating and stabilizing inhibitors of apoptosis, and that the shorter isoform somehow counters this action. While it cannot yet be demonstrated that modification of H2B contributes to these apoptotic effects, the findings in yeast ensure this remains a formal possibility.

**...And Dysregulation in Cancer**

**Dual nature of an H2B E3 ligase.** Disruption of the mechanisms that regulate development, including histone modification, may lead to uncontrolled cellular proliferation. With its reported roles in apoptosis, the cell cycle and DNA repair, disruption of the normal patterns of H2Bub1 may also be predicted to result in cancer. Importantly, multiple observations link RNF20 depletion to cancer progression: expression of RNF20 is reduced in metastatic as compared to benign prostate tumors, its promoter is hyper-methylated in several breast cancer cells, and it has been found to be mutated in colorectal tumors. Furthermore, knockdown of RNF20 in cell culture causes increased migration and anchorage-independent growth. This tumor suppressor role of RNF20 may be due to regulation of p53 function. It has been shown that H2Bub1 increases at the coding regions of genes involved in development.
p53-target genes upon their activation and RNF20 functions as a co-activator of p53-target genes. Knockdown of RNF20 in HeLa cells decreases p53 expression, with a resulting decrease in p53-mediated responses to genotoxic stress. This is accompanied by an increase in the expression of several proto-oncogenes and reduced expression of the tumor suppressor gene TP53BP1, suggesting that RNF20, and potentially H2Bub1, may restrain cellular proliferation through multiple pathways.

Conversely, other studies have alluded to a possible oncogenic role for RNF20. Increased Hox gene expression that results on upregulation of RNF20 may be predicted to contribute to oncogenesis. Methylation of H3K4 and H3K79 may be pivotal in this process, as hDot1L mis-targeting to Hoxa9 is implicated in leukaemogenesis. More directly, RNF20 knockdown in a breast cancer cell line reduces cell proliferation. However, it is also important to note that RNF20 also ubiquitylates a variant form of the transcriptional co-regulator Ebp1, thereby marking it for degradation. This Ebp1 isoform promotes cellular differentiation, and in accordance with a tumor suppressor function, various cancer cell lines reportedly exhibit reduced levels of this protein. Consequently, any oncogenic potential of RNF20 may be independent of H2Bub1.

Oncogenic nature of ubiquitin proteases. It is apparent that the role of RNF20 in the balance between cellular proliferation and arrest thereof is complex. Then, what of the enzymes that catalyze removal of the ubiquitin mark from H2B? In agreement with the relationship between ubiquitylation of H2B and tumor suppression, ubiquitin proteases that target H2B have been linked to oncogenesis. The gene encoding the ubiquitin protease USP22 is overexpressed as part of an eleven-gene cancer signature that can predict the future capacity of tumor cells for metastasis. USP22 is required for full activation of certain p53 and Myc target genes in human fibroblasts, and is required for malignant transformation by the latter (Table 1). Contrary to these findings, a recent study found that knockdown of USP22 resulted in an increase in the basal expression of p53 and its target p21 in a bladder cancer cell line, possibly as a result of a decrease in expression of Mdm2, a negative regulator of p53. This may be indicative of the effect of additional mutations in the cancer cell line used, highlighting the need for all findings to be confirmed in a variety of genetic backgrounds. USP22 is also required for full activation of certain nuclear receptors, including those of androgen and estrogen, and this has led others to speculate that USP22 may be culpable in the development of prostate and/or breast cancer.

Of interest, targets of a second oncogenic ubiquitin protease, USP7 (HAUSP; Table 1), include H2B, p53 and Mdm2; Mdm2 itself can bind and ubiquitylate H2B in vitro and in vivo. Furthermore, RNF20 is targeted to the promoter of the mdm2 gene in a p53-dependent manner, hinting at a complex network of H2B ubiquitylation in the control of cell cycle arrest. Finally, the gene encoding the human ubiquitin protease hUSP36 has been reported to be overexpressed in ovarian cancer tissue and cell lines as compared to healthy ovaries. While hUSP36 has not been reported to target H2B for deubiquitylation in vivo or in vitro, it shows homology to yeast Ubp10, implying a possible role for silencing of tumor suppressor genes through removal of the histone mark (Table 1).

Metabolism and carcinogens. It has been observed that H2B ubiquitylation is induced by glycolysis in yeast and human cells. Gao and Xu (2011) note that this may have implications for the metabolism of tumors, as cancer cells have an abnormally high glycolytic rate, and as such may have different H2Bub1 profiles. Perhaps of relevance is the observation that resveratrol, a polyphenol found in grapes, can prevent glycolysis in ovarian cancer cells, and was also recently shown to inhibit H2B ubiquitylation in multiple human cell lines. Knockdown of RNF20 in glioma cells inhibits their proliferation by inducing senescence, thereby recapitulating the effects of resveratrol on this cell line. Thus, it appears that dietary factors influence levels of H2Bub1, and this has implications for carcinogenesis.

Certain metal compounds, namely nickel chloride and cobalt chloride, are able to increase levels of H2Aub1 and H2Bub1 in human cancer cell lines. Cobalt chloride induced a similar increase in H2Bub1 and H2Aub1 in various human cell lines, as well as disrupting expression of several hundred genes, including many oncogenes. It was demonstrated in vitro that nickel and cobalt ions are able to prevent histone deubiquitylation, without affecting ubiquitylation per se. This effect appears to be specific to histones, as levels of ubiquitylated non-histone proteins remain unchanged. While any direct effect of such chemically-induced reductions in histone deubiquitylation on carcinogenesis cannot be inferred, these studies further suggest that altered cellular profiles of H2Bub1 may be indicative of increased susceptibility to cancer development.

Fundamental Questions

In this review, we have discussed the current understanding of the involvement of H2Bub1 in developmental processes; an overview is provided in Figure 5. We believe that the role of H2Bub1 in many of these cases is through gene-differential effects on transcriptional activation, but cannot preclude the possibility that gene expression may be affected at additional levels. Indeed, a flurry of studies demonstrated that certain histone modifications show differential patterns of enrichment at introns and exons, independently of nucleosome occupancy and transcriptional activity. Some of these histone marks have already been demonstrated to regulate alternative splicing decisions; specific depletion of H3K4me3 reduces the rate of pre-mRNA splicing, and its overexpression results in increased inclusion of an alternatively spliced exon of the FGFR2 gene, in cells in which its inclusion is normally low. Indicating a possible relationship between H2Bub1 and splicing, H2Bub1 is enriched in the 5′ introns of human genes. Perhaps of relevance is the finding that the protein WAC interacts with RNF20/40, and is required for ubiquitylation of H2B in human cell culture. Transfected WAC co-localizes with SC35, a splicing factor, in mammalian cell culture, and an apparent fusion protein with regions homologous to both proteins exists in fruit flies. Consequently, perhaps WAC is important for integrating H2B ubiquitylation and RNA splicing mechanisms.
While this review has concentrated on the development consequences of H2B ubiquitylation, it would be remiss of us not to acknowledge that the effect of H2B ubiquitylation on chromatin remains vague. Recent studies present apparently contradictory conclusions as to the nature of H2Bub1. H2B K123R yeast strains exhibit increased histone solubility, suggesting H2Bub1 stabilizes the nucleosome, and yet synthetic nucleosome arrays containing H2Bub1 are less compact than those containing unmodified H2B.111,112 This latter finding is seemingly more in line with the observation that chemically-induced relaxation of chromatin rescues the RAD51 recruitment defect of RNF20 deficient cells.39 How then do we reconcile defects in chromatin compaction with increased nucleosome stability? We might argue that ubiquitylation of H2B initially disrupts higher-order chromatin compaction, perhaps thereby exposing binding sites for proteins that stabilize nucleosomes.

The findings of another recent report suggest that H2Bub1 may not alter chromatin compaction by virtue of its added bulk, but perhaps recruits effector proteins directly.113 While genes that are suppressed by RNF20 reside within regions of compact chromatin, knockdown of RNF20 has a global effect on the association between chromatin and TFIIIS.113 This implies that H2Bub1 does not result in gene suppression by contributing to the closed state; rather, by preventing TFIIIS recruitment, the cell is unable to relieve the transcriptional blocks that occur frequently at closed chromatin, and this underlies gene suppression.113 This begs the question: how does RNF20 activity recruit TFIIIS? Heretofore, no protein has been determined to interact directly with H2Bub1; however absence of evidence is not the same as evidence of absence.

In summary, understanding the requirement of H2Bub1 for development in higher eukaryotes depends largely on comprehending how it regulates gene expression during cell proliferation and differentiation; we anticipate that advances in high-throughput ChIP-sequencing will facilitate the generation of H2Bub1 profiles for multiple cell lines and tissues, thereby allowing us to determine the target genes at different stages.114 This has already been performed for Drosophila melanogaster; through the modENCODE (model organism ENCYclopedia Of DNA Elements) project, H2Bub1 patterns for the fruit fly genome have been determined in several cell lines, revealing that it is enriched throughout the body of transcribed genes. This pattern was then used to successfully identify previously unannotated genes.115 Additionally, elucidation of the role of human H2Bub1 in normal development will aid us in understanding how disruption of its regulation results in cancer, potentially leading to new therapeutical strategies. As discussed, this single histone modification is important for a plethora of biological processes, and yet its mechanism of action remains poorly understood, despite recent findings.112 With the progression of the modENCODE project, we believe that we stand at the dawn of a comprehensive understanding of the role of chromatin structure and histone modifications in the stereotyped programming of development, and the integral requirement of H2Bub1 therein.

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References


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