Short Article

The Histone H2B Monoubiquitination Regulatory Pathway Is Required for Differentiation of Multipotent Stem Cells

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SUMMARY

Extensive changes in posttranslational histone modifications accompany the rewiring of the transcriptional program during stem cell differentiation. However, the mechanisms controlling the changes in specific chromatin modifications and their function during differentiation remain only poorly understood. We show that histone H2B monoubiquitination (H2Bub1) significantly increases during differentiation of human mesenchymal stem cells (hMSCs) and various lineage-committed precursor cells and in diverse organisms. Furthermore, the H2B ubiquitin ligase RNF40 is required for the induction of differentiation markers and transcriptional reprogramming of hMSCs. This function is dependent upon CDK9 and the WAC adaptor protein, which are required for H2B monoubiquitination. Finally, we show that RNF40 is required for the resolution of the H3K4me3/H3K27me3 bivalent poised state on lineage-specific genes during the transition from an inactive to an active chromatin conformation. Thus, these data indicate that H2Bub1 is required for maintaining multipotency of hMSCs and plays a central role in controlling stem cell differentiation.

INTRODUCTION

Both embryonic development and tissue homeostasis require the expansion and differentiation of multipotent self-renewing stem cells. Stem cells can differentiate into a variety of cell types depending upon the stimulus provided. A deeper understanding of the molecular mechanisms determining cell-type-specific differentiation may provide therapeutic opportunities for treating a variety of pathological conditions.

Stem cells exhibit specific gene expression signatures which dictate cell fate during differentiation (Fisher and Fisher, 2011). A unique pattern of posttranslational histone modifications in stem cells governs gene expression patterns and differentiation potential (Fisher and Fisher, 2011; Meissner, 2010). Genome-wide studies have revealed that many developmental genes exhibit "bivalent" chromatin domains displaying both "active" (H3K4me3) and "repressive" (H3K27me3) histone modifications (Azuara et al., 2006; Bernstein et al., 2006). This "poised" state is proposed to allow for correct temporal gene expression.

The transcriptional apparatus and chromatin modifications exhibit a high degree of crosstalk. For example, phosphorylation of Ser2 (P-Ser2) within the heptapeptide repeat of the RNA polymerase II (RNAPII) C-terminal domain (CTD) provides a platform for recruiting the WW domain containing adaptor protein with coiled coil (WAC) (Zhang and Yu, 2011). In turn, WAC recruits the RNF20/RNF40 ubiquitin ligase complex which monoubiquitinates H2B (Kim et al., 2009; Zhu et al., 2005). Knockdown of either CDK9 (Pirngruber et al., 2009b) or WAC (Zhang and Yu, 2011) elicits an effect on H2Bub1 similar to that of RNF20 or RNF40 depletion.

Consistent with its dependence upon the elongating form of RNAPII, H2Bub1 is primarily associated with the transcribed regions of active genes (Minsky et al., 2008) where it facilitates transcriptional elongation (Pavri et al., 2006; Prenzel et al., 2011). However, despite its association with active genes, H2Bub1 is only required for the transcription of a subset of inducible genes (Prenzel et al., 2011; Shema et al., 2008). Consistent with a specific regulation of tumor-relevant transcriptional pathways, H2Bub1 is a proposed tumor suppressor whose levels decrease during tumor progression (Johnsen, 2012; Prenzel

et al., 2011; Shema et al., 2008). The inverse relationship between differentiation status and malignancy may indicate that decreased H2Bub1 in advanced tumors leads to a less differentiated phenotype. However, the role of H2Bub1, its modifying enzymes, and upstream regulators in controlling cell-fate determination in mammalian systems remains unexplored.

Here we demonstrate that H2Bub1 levels increase in diverse cell differentiation systems. Perturbation of H2B monoubiquitination via depletion of its ubiquitin ligases RNF40 and RNF20, or upstream regulators CDK9 and WAC impairs stem cell differentiation. RNF40 depletion affects multipotency by preventing the resolution of bivalent histone marks on lineage-specific genes. These results uncover a previously unknown function of H2Bub1 and its upstream regulatory pathway in stem cell differentiation and shed light into the mechanism by which its loss may increase malignant potential.

RESULTS

H2Bub1 Increases during Cellular Differentiation

We previously reported decreased H2Bub1 levels during malignant progression (Prenzel et al., 2011). Given the inverse correlation between tumor differentiation status and malignancy, we hypothesized that H2Bub1 may increase during normal cellular differentiation. We therefore examined H2Bub1 levels in various differentiation systems including telomerase-immortalized human mesenchymal stem cells (hMSCs) (Simonsen et al., 2002) differentiated to either the osteoblast (Figure 1A) or adipocyte lineages (Figure 1B), human fetal osteoblast (hFOB) cells (Figure 1C), mouse neurospheres (Figure 1D, see Figure S1A available online), and oligodendrocytes (Figure 1E). In each of the investigated mammalian model systems, H2Bub1 levels increased concomitantly with the induction of lineage specification which was accompanied by increased RNF40 protein levels in hMSC (Figure S1B). In contrast, the levels of H2A monoubiquitination remained constant during hMSC differentiation (Figure S1C).

In order to determine whether the increase in H2Bub1 levels during cellular differentiation is conserved across species, we performed immunofluorescence analyses of H2Bub1 in the *Drosophila melanogaster* ovary. As observed in undifferentiated mammalian cells, H2Bub1 was undetectable in *Drosophila* germline stem cells (characterized by the presence of phospho-Mad; Figure 1F). In contrast, differentiated cystoblasts displayed readily detectable H2Bub1 levels. Thus, increased H2Bub1 levels during cellular differentiation are common to multiple differentiation programs and across species.

RNF40 Knockdown Impairs hMSC Differentiation

To test whether H2Bub1 is required for differentiation, we examined the effects of RNF40 depletion on hMSC differentiation. As assessed by staining for differentiated osteoblasts (alkaline phosphatase) or adipocytes (oil red O), we observed a clear decrease in both differentiation models following RNF40 knockdown with independent RNF40 siRNAs (Figures 2A and 2B; Figures S2A–S2F). Consistently, no lipid droplet formation was observed by electron microscopy in RNF40-depleted cells grown under adipocyte differentiating conditions (Figure S2G).

RNF40 Knockdown Impairs Differentiation-Regulated Transcription

In order to determine the extent of the effects elicited by RNF40 knockdown on hMSC differentiation, we performed siRNA knockdown and transcriptome-wide gene expression microarray analyses of undifferentiated hMSCs and cells differentiated to the adipocyte or osteoblast lineages for 2 or 5 days. RNF40 knockdown had little or no effect on gene expression in undifferentiated hMSCs (Figure S2J). Strikingly, the differentiation-induced gene expression changes were significantly impaired following RNF40 knockdown (Figures 2C and 2D) without affecting the expression of the osteoblast and adipocyte-regulatory factors *RUNX2*, *CEBPB*, and *CEBPG* (Figures S2H and S2I). The effects of RNF40 knockdown were evident for common (Figure S2K) as well as lineage-specific (Figures S2L and S2M) transcriptional programs. Pathways significantly enriched in gene sets differentially regulated in each differentiation program and by RNF40 depletion (Table S1) include focal adhesion (Figures S2N–S2Q), cell cycle (Figures S2R–S2U), adipocytokine signaling (Figures S2V and S2W), and pathways in cancer (Figures S2X–S2AA). Microarray data were verified on a number of osteoblast (*BGLAP*, *ALPL*, and *G6PD*)- and adipocyte (*PDK4*, *PPARG*, and *RASD1*)-specific genes (Figures 2E and 2F, respectively). Consistent with the obligate heterodimeric function of RNF20 and RNF40, RNF20 knockdown also decreased H2Bub1 levels and impaired the induction of osteoblast- and adipocyte-specific gene expression (Figures S2AB–S2AE). Importantly, RNF40 depletion did not affect mRNA levels of other pathway components (CDK9, WAC, RNF20; Figures S2AF and S2AG).

CDK9 Controls hMSC Differentiation

Since CDK9 controls the global and gene-specific levels of H2Bub1 (Pirngruber et al., 2009b; Shchebet et al., 2012), we hypothesized that CDK9 may also be required for hMSC differentiation by directing RNF40-dependent H2B monoubiquitination through P-Ser2 RNAPII CTD. Consistent with this notion, the levels of P-Ser2 increased during hMSC differentiation (Figure S3A).

We next examined the effects of CDK9 knockdown on hMSC differentiation. Similar to the effects of RNF40 knockdown, adipocyte differentiation and gene expression (Figures 3C and 3D, Figure S3F) were impaired following CDK9 knockdown, as was the expression of the osteoblast marker genes *BGLAP* and *G6PD* (Figure 3B) and global H2Bub1 levels (Figures S3B and S3C). Surprisingly, the induction of alkaline phosphatase activity (Figure 3A, Figure S3E) and *ALPL* gene expression (Figure 3B) was increased following CDK9 knockdown. Since alkaline phosphatase is also a marker of pluripotency, these results may indicate a reversion to an earlier stem cell phenotype. These effects were not due to impaired expression of H2Bub1 pathway regulators (Figures S3G and S3H). Thus, we conclude that CDK9 knockdown prevents hMSC differentiation irrespective of cell lineage.

WAC Knockdown Phenocopies RNF40 Depletion

WAC functionally links CDK9 and H2Bub1 during transcription by recruiting the RNF20/RNF40 complex to P-Ser2 RNAPII
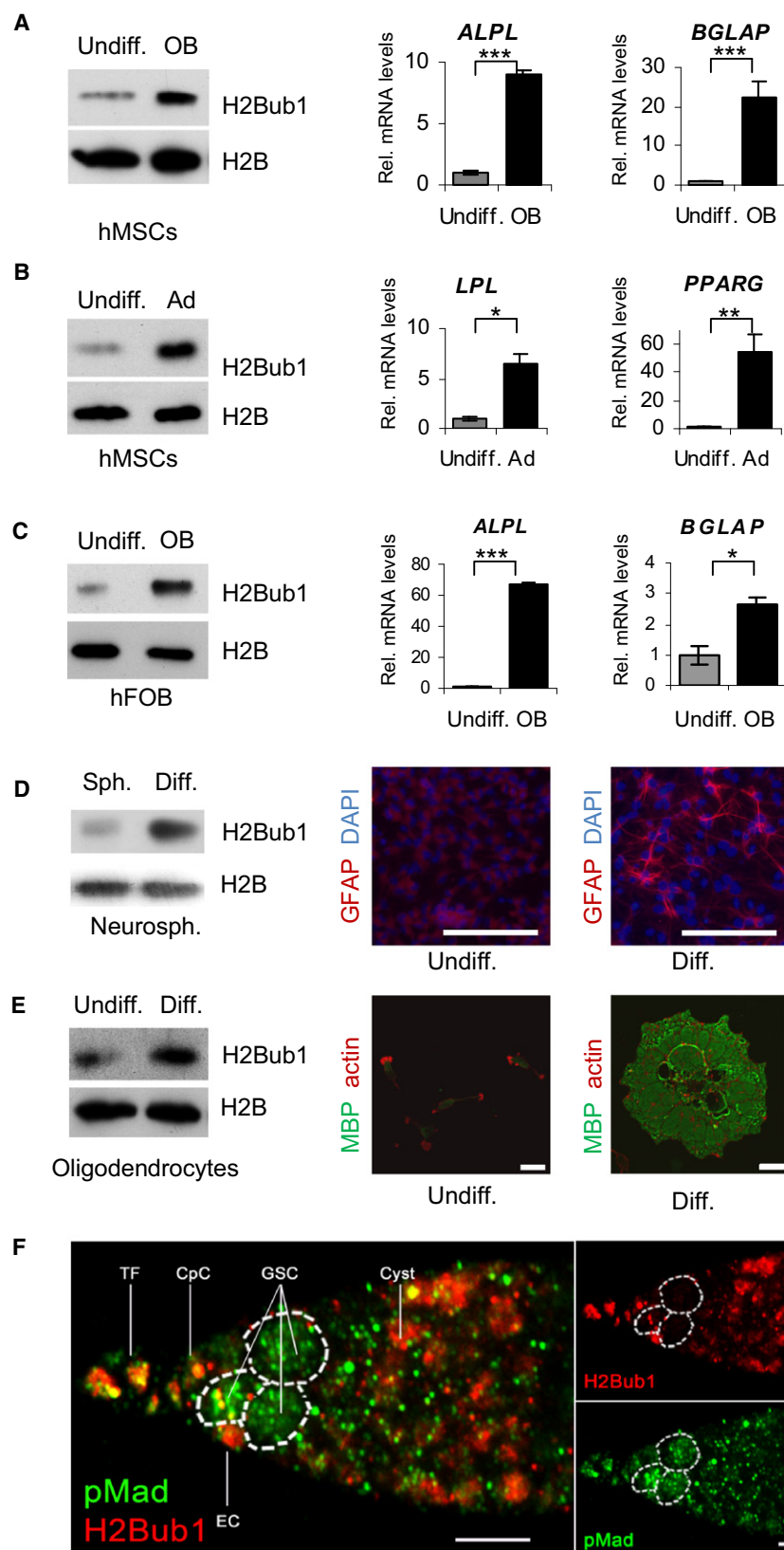
Figure 1. H2Bub1 Levels Increase upon Differentiation

(A and B) Immortalized human mesenchymal stem cells (hMSCs) were induced to differentiate into the osteoblast (A) or adipocyte (B) lineages for 5 days. Protein extracts were analyzed by western blot using antibodies against H2Bub1 or H2B (left panel). The expression of the osteoblast (*ALPL*, *BGLAP*; A) or adipocyte (*LPL*, *PPARG*; B) marker genes was verified by qRT-PCR. Gene expression was normalized to a control gene (*HNRNPK*) and the undifferentiated condition and expressed as "relative mRNA levels." Mean ± SD, n = 3. Statistical analysis, *p < 0.05; **p < 0.01; ***p < 0.001.

(C) Human fetal osteoblasts (hFOBs) were differentiated and analyzed by western blot for H2Bub1 and H2B as in (A). Induction of osteoblast marker genes (*ALPL*, *BGLAP*) was verified as in (A). Mean ± SD, n = 3.

(D) Mouse neurospheres were induced to differentiate and analyzed by western blot as in (A). Differentiation to glial cells was examined by immunofluorescence staining of GFAP (red). Nuclei were costained with DAPI (blue).

(E) Mouse oligodendrocytes were differentiated for 5 days and analyzed for H2Bub1 and H2B levels by western blot. Differentiation was verified by immunofluorescence staining against myelin basic protein (MBP, green) and actin (red).

(F) Immunofluorescence analysis of *Drosophila melanogaster* ovary stained for H2Bub1 and phosphorylated Mad (pMad; marker of proliferating stem cells). TF, terminal filament cells; CpC, cap cells; GSC, germline stem cells; Cyst, cystoblast; EC, escort cells.
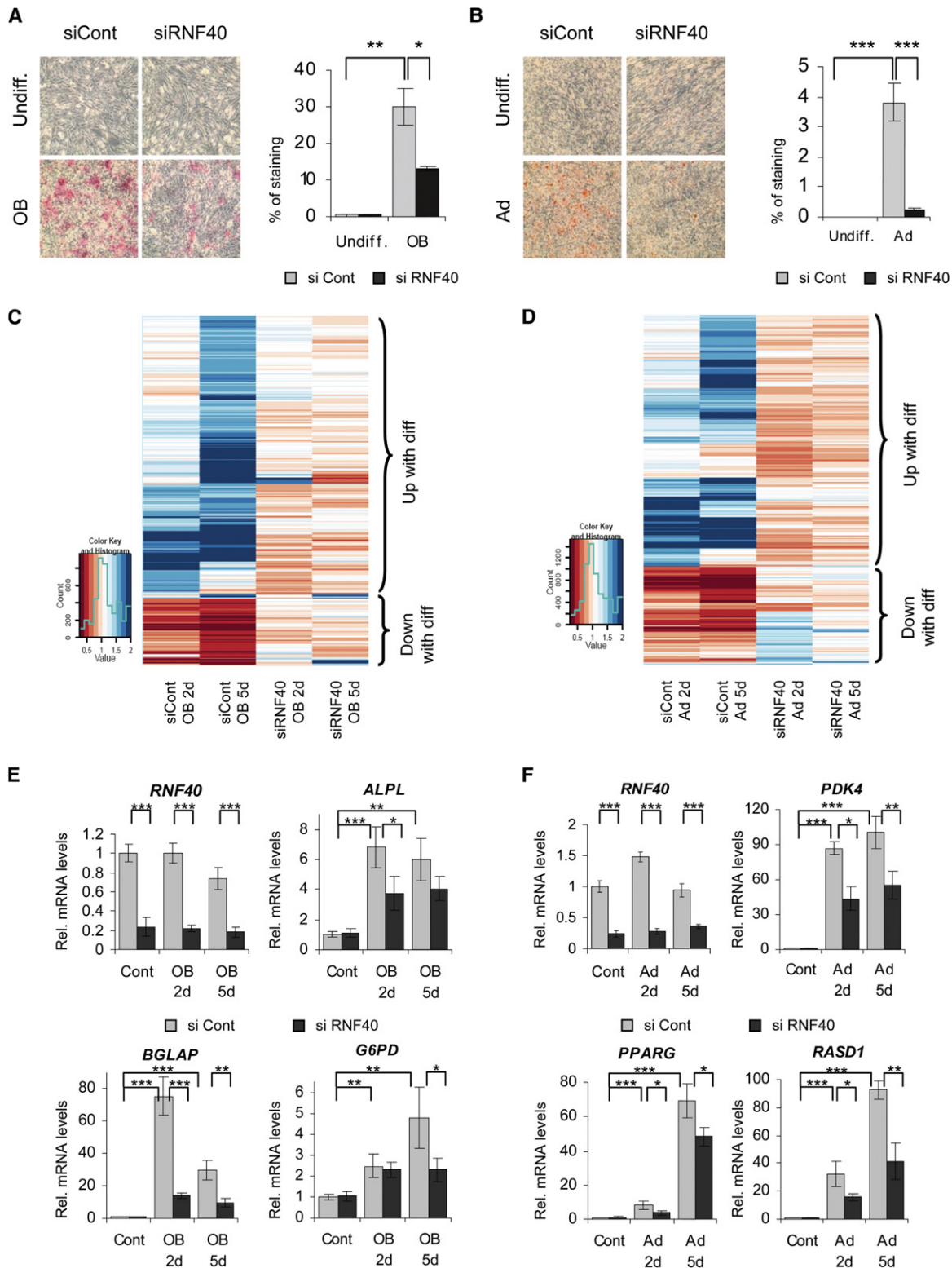
Figure 2. RNF 40 Is Required for hMSC Differentiation

(A and B) hMSCs were transfected with control and RNF40 siRNAs and induced to differentiate into osteoblasts (A) or adipocytes (B) for 5 days. Differentiation was verified by alkaline phosphatase (A) or oil red O (B) staining (left). The stained area was quantified using ImageJ software and displayed as percentage of area (right). Mean ± SD, n = 3.

CTD (Zhang and Yu, 2011). Consistent with this report, WAC was required for the stability of the RNF20/40 complex (Figures S3I and S3J) and maintaining H2Bub1 levels (Figures S3K–S3N). Importantly, WAC knockdown impaired osteoblast (Figure 3E and Figure S3O) and adipocyte (Figure 3G and Figure S3P) differentiation and the induction of marker gene expression (Figures 3F and 3H). While RNF20, RNF40, and WAC protein levels were dependent upon one another (Figures S3I and S3J), their mRNA levels were unaffected by WAC knockdown (Figures S3Q and S3R). These results provide a mechanistic explanation for how CDK9 directs H2Bub1 during differentiation through RNAPII CTD phosphorylation and recruitment of the WAC/RNF20/RNF40 ubiquitin ligase complex. Perturbation of any component similarly impairs stem cell differentiation.

RNF40 Is Required for Resolution of Bivalency

In order to test whether the effects of RNF40, RNF20, CDK9, and WAC depletion on hMSC differentiation may be a direct effect of H2Bub1, we performed chromatin immunoprecipitation analyses on the adipocyte-specific genes *PDK4*, *PPARG*, and *RASD1* as well as positive (*GAPDH*) and negative (*TFF1*) control genes. Indeed, all three lineage-specific genes showed increased levels of H2Bub1 during differentiation (Figure 4A). Importantly, the levels of H2Bub1 were constantly present on the *GAPDH* gene and decreased following RNF40 knockdown but were constitutively absent from the silenced *TFF1* gene.

Embryonic stem cells and some lineage-committed stem cells display a unique combination of both activating (H3K4me3) and repressing (H3K27me3) histone modifications on many lineage-specific genes (Collas, 2010; Fisher and Fisher, 2011). During differentiation, bivalency on adipocyte-specific genes is resolved in favor of an active state in which H3K4me3 is retained while H3K27me3 is lost (Collas, 2010; Noer et al., 2009). Perturbations in the resolution of bivalency impair the inducibility of lineage-specific genes during differentiation. The analysis of genome-wide chromatin immunoprecipitation-sequencing data (ChIP-Seq) from human embryonic stem cells (hESCs) and normal adipose nuclei confirmed that the investigated adipocyte-specific genes (*PDK4*, *PPARG*, *RASD1*) are all bivalent in hESC and lose their bivalency in favor of H3K4me3 during terminal adipocyte differentiation (Figure 4B).

Given the established dependence of H3K4me3 on H2Bub1 in some systems (Kim et al., 2009; Pirngruber et al., 2009a; Zhu et al., 2005), we hypothesized that the impaired differentiation observed following RNF40 knockdown (with no major effects on the global gene expression profile) may be due to changes in the bivalency status of differentiation-induced genes. Surprisingly, while adipocyte-specific genes (*PDK4*, *PPARG*, *RASD1*) exhibit significant levels of both H3K4me3 and H3K27me3 in the undifferentiated state (Figures 4C and 4D), neither the global (Figure S4) nor the gene-associated levels of H3K4me3 were reduced following RNF40 knockdown (Figure 4C). This supports

the findings that H3K4me3 may be independent of H2Bub1 in some systems (Shema et al., 2008; Vethantham et al., 2012). As previously reported (Noer et al., 2009), bivalency was resolved in favor of H3K4me3 on adipocyte-specific genes during differentiation (Figure 4D). In contrast, H3K27me3 was retained on bivalent adipocyte-specific genes following RNF40 knockdown. Thus RNF40-dependent H2B monoubiquitination appears to be dispensable for H3K4me3 but essential for the resolution of bivalency on lineage-specific genes.

DISCUSSION

In order to obtain a clearer understanding of the roles of specific chromatin modifications in the transition from stem cells into a differentiated phenotype, we investigated the effects of perturbing H2Bub1 levels during cellular differentiation. We show that both global and lineage-specific gene-associated H2Bub1 levels increase during hMSC differentiation. Although a recent study reported a decrease in H2Bub1 during myoblast differentiation (Vethantham et al., 2012), our data suggest that H2Bub1 levels increase in multiple diverse differentiation systems. Furthermore, this process is evolutionarily conserved. Notably, in addition to the investigated mouse and human cell culture differentiation models, we demonstrate that H2Bub1 is suppressed in stem cells in vivo. Although a suppression of H2Bub1 was previously suggested to be essential for stem cell maintenance in *Drosophila* (Buszczak et al., 2009), we now show that H2Bub1 levels indeed increase during differentiation and that its modifying enzymes and upstream regulators are essential for the differentiation process.

We have demonstrated that CDK9 activity is essential for the induction of lineage-specific gene expression. Data in *Drosophila* and *C. elegans* support this observation, since a suppression of CDK9 activity is required for stem cell maintenance (Batchelder et al., 1999; Hanyu-Nakamura et al., 2008; Zhang et al., 2003). Furthermore, it has been shown that CDK9 activity is required for differentiation in several mammalian cell systems, including adipocyte (Iankova et al., 2006), myoblast (Simone et al., 2002), monocyte (Yu et al., 2006), and cardiomyocyte differentiation (Kaichi et al., 2011). Given that a large fraction of unexpressed genes in ESCs exhibit paused RNAPII lacking P-Ser2 (Guenther et al., 2007), suppression of CDK9 activity may be a general mechanism employed for maintaining a stem cell state. Like bivalent chromatin domains, the presence of a paused RNAPII is expected to allow the timely induction of differentiation-related genes.

Specific chromatin states play an essential role in controlling pluripotency, and dynamic changes in these states are required for proper lineage-specific differentiation (Fisher and Fisher, 2011; Meissner, 2010). We have confirmed that adipocyte-specific genes exist in a bivalent chromatin state in multipotent hMSCs. In parallel with the global increase in H2Bub1 levels,

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(C and D) Transcriptome-wide gene expression microarray analysis of hMSC induced to differentiate into the osteoblast (C) or adipocyte (D) lineages for 2 or 5 days after RNF40 knockdown. The heatmaps indicate the fold changes in mRNA levels in the various conditions relative to the undifferentiated control transfected cells. Color code indicates downregulated genes in red and upregulated genes in blue. Mean values, n = 3.
(E and F) qRT-PCR analyses verify the knockdown of RNF40 in the RNA samples utilized for microarray analyses in (C and D) as well as the changes in gene expression of selected osteoblast (*ALPL*, *BGLAP*, *G6PD*; E) and adipocyte (*PDK4*, *PPARG*, *RASD1*; F) marker genes. Mean ± SD, n = 3.
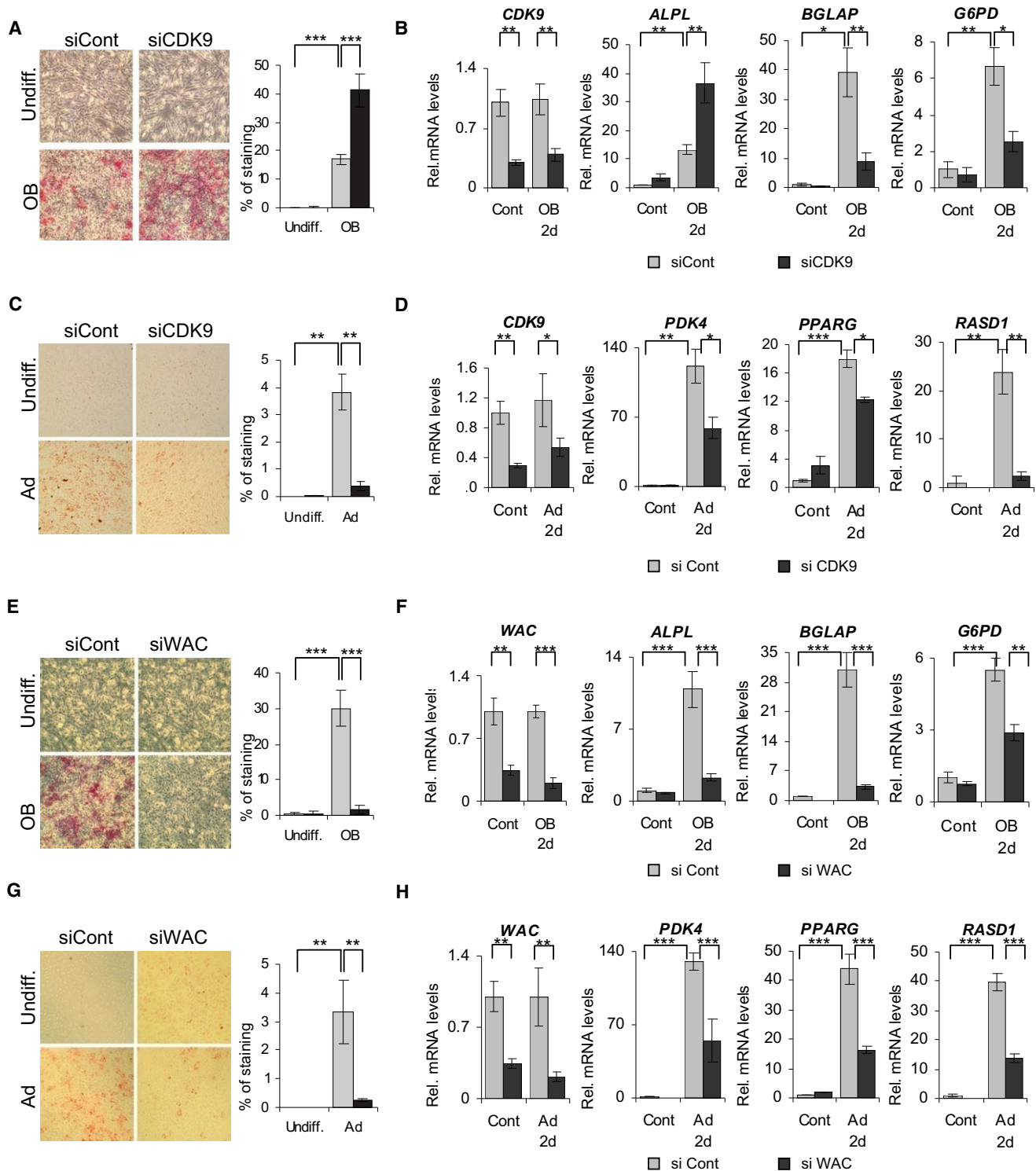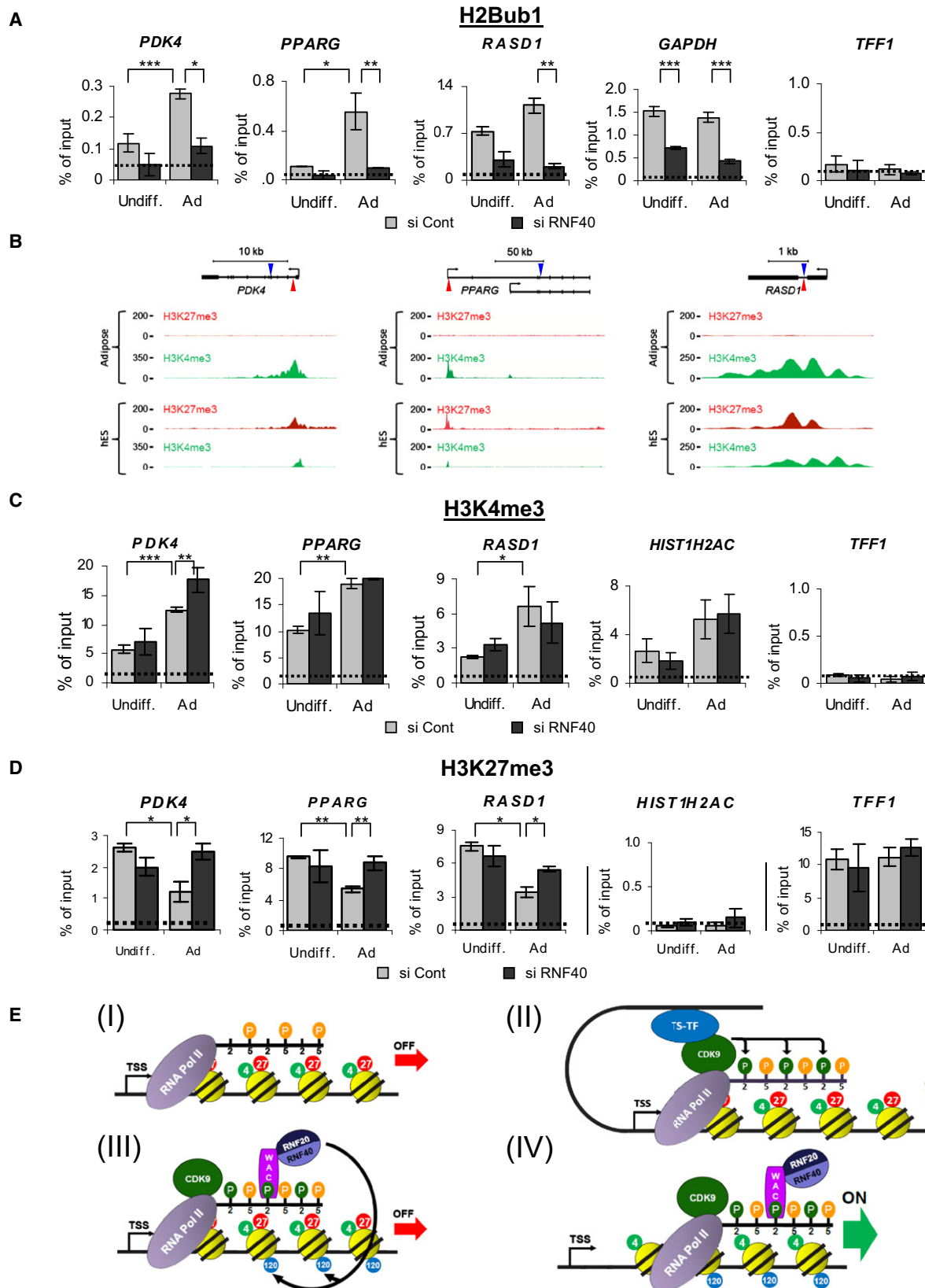
Figure 3. CDK9 and WAC Direct H2Bub1 during Differentiation

(A–H) hMSCs transfected with siRNAs against CDK9 (A–D) or WAC (E–H) were induced to differentiate into the osteoblast (A and B, E and F) or adipocyte (C and D, G and H) lineages. Cells were stained for alkaline phosphatase activity (A and E) or oil red O (C and G) for osteoblast and adipocyte lineages, respectively. Staining was quantified as in Figures 2A and 2B. Mean ± SD, n = 3. (B, D, F, and H) The knockdown efficiency and the effects on osteoblast- (*ALPL*, *BGLAP*, *G6PD*; B and F) and adipocyte-specific (*PDK4*, *PPARG*, *RASD1*; D and H) gene expression were verified by qRT-PCR and analyzed as in Figures 2E and 2F following 2 days of differentiation. Mean ± SD, n = 3.

this bivalency is resolved, and these genes become fully active and exhibit a decrease in H3K27me3 while retaining H3K4me3. In our model, tissue-specific transcription factors promote differentiation in a CDK9-dependent manner by directing H2Bub1 (Figure 4E). In turn, H2Bub1 (or possibly RNF40 directly) likely directs dynamic changes in chromatin structure which facilitate the demethylation of H3K27me3 during the transition from a repressive to an active chromatin state. Consistently, we and others have shown that RNF20 and RNF40 cooperate with the FACT histone chaperone complex to stimulate histone exchange and facilitate transcriptional elongation (Pavri et al., 2006; Prenzel et al., 2011). Consistent with a CDK9→WAC→RNF20/RNF40→H2Bub1→FACT regulatory circuit, the presence of both H2Bub1 and FACT on target genes depends upon CDK9 activity rather than transcriptional activity per se (Gomes et al., 2006; Pirngruber et al., 2009b). Interestingly, the chromatin remodeling factor CHD1 is required for pluripotency (Gaspar-Maia et al., 2009) and was independently identified in complexes containing CDK9 (Park et al., 2010) and FACT (Kelley et al., 1999). Furthermore, a recent study identified a functional interaction between H2Bub1 and Chd1 in yeast (Lee et al., 2012). Moreover, mammalian CHD1 interacts directly with H3K4me3 through its chromodomain (Sims et al., 2007) but is excluded from bivalent domains (Gaspar-Maia et al., 2009). Thus, the maintenance of H3K27me3 at bivalent genes following RNF40 knockdown may function in part by preventing CHD1 recruitment.

In conclusion, our data establish a role for CDK9, WAC, and RNF40 in directing H2B monoubiquitination and transcriptional reprogramming during stem cell differentiation. This effect appears to be due to dynamic changes in chromatin structure during differentiation, which facilitate a transition from an inactive to an active chromatin state. In keeping with this, we observed maintenance of H3K27me3 levels on bivalent adipocyte-specific genes following RNF40 depletion. Thus these data place H2Bub1 and its regulatory pathway as central players in cell-fate determination and underscore the importance of dynamic changes in chromatin structure during cellular differentiation.

EXPERIMENTAL PROCEDURES

Cell Culture, Differentiation, and Knockdowns

hMSC and hFOB1.17 cells were cultured and differentiated as previously done (Simonsen et al., 2002; Harris et al., 1995) and are described together with staining procedures and the generation, culture, and differentiation of neurospheres and oligodendrocytes in the Supplemental Experimental Procedures. Knockdown procedures and siRNAs utilized are listed in the Supplemental Information.

Western Blot Analysis, RNA Isolation, qRT-PCR, ChIP

Western blot analysis was performed with antibodies and dilutions listed in the Supplemental Information. RNA isolation, reverse transcription, ChIP, and quantitative RT-PCR were performed as described (Prenzel et al., 2011) with the modifications listed in the Supplemental Experimental Procedures. ChIP-Seq results were obtained from the NIH Roadmap Epigenomics Project (Bernstein et al., 2010), visualized using the UCSC Genome Browser, and used under permission from the NIH Epigenome Mapping Center at Broad Institute.

Microscopy

For analyzing H2Bub1 levels in vivo, ovaries from OregonR *Drosophila* were fixed and stained as described in the Supplemental Experimental Procedures.

Microarray Studies

Whole-genome gene expression analysis was performed by the Vancouver Prostate Centre Laboratory for Advanced Genome Analysis (Vancouver, Canada) using the Illumina human HT-12 v4 beadchip. Gene expression data were analyzed as previously described (Prenzel et al., 2011). Gene set pathway analyses were performed as described in the Supplemental Experimental Procedures. All gene expression data will be made publicly available through the GEO repository.

ACCESSION NUMBERS

The Gene Expression Omnibus (GEO) accession numbers for the ES and adipose nuclei ChIP-Seq reported in this paper are GSM669889, GSM669897, GSM669925, and GSM669930. The GEO accession number for the gene expression microarray analyses reported in is paper is GSE38173.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, one table, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at doi:10.1016/j.molcel.2012.05.022.

Figure 4. RNF40 Expression Is Required for the Resolution of Bivalent Chromatin Marks

(A) ChIP analysis of H2Bub1 on adipocyte-regulated genes (*PDK4*, *PPARG*, and *RASD1*) upon RNF40 knockdown. hMSCs were differentiated to adipocytes for 2 days following RNF40 depletion for 24 hr. *GAPDH* serves as a positive control for an active gene displaying H2Bub1, and *TFF1* serves as a control for an inactive gene with no detectable H2Bub1. Background (IgG) levels are indicated as dotted lines. Mean ± SD, n = 3.

(B) Human ESCs demonstrate bivalent domains containing both H3K4me3 and H3K27me3 on the adipocyte-regulated genes *PDK4*, *PPARG*, and *RASD1* while this bivalency is resolved in favor of H3K4me3 in adipose nuclei. Red triangles indicate amplicons used for detection of bivalent domains; blue trianges indicate amplicons used for detection of H2Bub1 within the transcribed region.

(C and D) H3K4me3 (C) and H3K27me3 (D) occupancy on adipocyte-regulated genes as well as one active gene (HIST1H2AC) and one repressed (TFF1) control gene. ChIP analysis was performed in hMSCs as in (A) following RNF40 depletion. Mean ± SD, n = 3.

(E) Model of the role of H2Bub1 in differentiation. "4" in green circles, H3K4me3; "27" in red circles, H3K27me3; "120" in blue circles, H2Bub1; "P" in orange circles, Ser5 phosphorylation of CTD; "P" in green circles, Ser2 phosphorylation of CTD; TSS, transcription start site.

REFERENCES

Azura, V., Perry, P., Sauer, S., Spivakov, M., Jorgensen, H.F., John, R.M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M., and Fisher, A.G. (2006). Chromatin signatures of pluripotent cell lines. Nat. Cell Biol. 8, 532–538.

Batchelder, C., Dunn, M.A., Choy, B., Suh, Y., Cassie, C., Shim, E.Y., Shin, T.H., Mello, C., Seydoux, G., and Blackwell, T.K. (1999). Transcriptional repression by the Caenorhabditis elegans germ-line protein PIE-1. Genes Dev. 13, 202–212.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326.

Bernstein, B.E., Stamatoyannopoulos, J.A., Costello, J.F., Ren, B., Milosavljevic, A., Meissner, A., Kellis, M., Marra, M.A., Beaudet, A.L., Ecker, J.R., et al. (2010). The NIH Roadmap Epigenomics Mapping Consortium. Nat. Biotechnol. 28, 1045–1048.

Buszczak, M., Paterno, S., and Spradling, A.C. (2009). Drosophila stem cells share a common requirement for the histone H2B ubiquitin protease scrawny. Science 323, 248–251.

Collas, P. (2010). Programming differentiation potential in mesenchymal stem cells. Epigenetics 5, 476–482.

Fisher, C.L., and Fisher, A.G. (2011). Chromatin states in pluripotent, differentiated, and reprogrammed cells. Curr. Opin. Genet. Dev. 21, 140–146.

Gaspar-Maia, A., Alajem, A., Polesso, F., Sridharan, R., Mason, M.J., Heidersbach, A., Ramalho-Santos, J., McManus, M.T., Plath, K., Meshorer, E., and Ramalho-Santos, M. (2009). Chd1 regulates open chromatin and pluripotency of embryonic stem cells. Nature 460, 863–868.

Gomes, N.P., Bjerke, G., Llorente, B., Szostek, S.A., Emerson, B.M., and Espinosa, J.M. (2006). Gene-specific requirement for P-TEFb activity and RNA polymerase II phosphorylation within the p53 transcriptional program. Genes Dev. 20, 601–612.

Guenther, M.G., Levine, S.S., Boyer, L.A., Jaenisch, R., and Young, R.A. (2007). A chromatin landmark and transcription initiation at most promoters in human cells. Cell 130, 77–88.

Hanyu-Nakamura, K., Sonobe-Nojima, H., Tanigawa, A., Lasko, P., and Nakamura, A. (2008). Drosophila Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells. Nature 451, 730–733.

Harris, S.A., Enger, R.J., Riggs, B.L., and Spelsberg, T.C. (1995). Development and characterization of a conditionally immortalized human fetal osteoblastic cell line. J. Bone Miner. Res. 10, 178–186.

Iankova, I., Petersen, R.K., Annicotte, J.S., Chavey, C., Hansen, J.B., Kratchmarova, I., Sarruf, D., Benkirane, M., Kristiansen, K., and Fajas, L. (2006). Peroxisome proliferator-activated receptor gamma recruits the positive transcription elongation factor b complex to activate transcription and promote adipogenesis. Mol. Endocrinol. 20, 1494–1505.

Johnsen, S.A. (2012). The enigmatic role of H2Bub1 in cancer. FEBS Lett. 586, 1592–1601.

Kaichi, S., Takaya, T., Morimoto, T., Sunagawa, Y., Kawamura, T., Ono, K., Shimatsu, A., Baba, S., Heike, T., Nakahata, T., and Hasegawa, K. (2011). Cyclin-dependent kinase 9 forms a complex with GATA4 and is involved in the differentiation of mouse ES cells into cardiomyocytes. J. Cell. Physiol. 226, 248–254.

Kelley, D.E., Stokes, D.G., and Perry, R.P. (1999). CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. Chromosoma 108, 10–25.

Kim, J., Guermah, M., McGinty, R.K., Lee, J.S., Tang, Z., Milne, T.A., Shilatifard, A., Muir, T.W., and Roeder, R.G. (2009). RAD6-mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell 137, 459–471.

Lee, J.S., Garrett, A.S., Yen, K., Takahashi, Y.H., Hu, D., Jackson, J., Seidel, C., Pugh, B.F., and Shilatifard, A. (2012). Codependency of H2B monoubiquitination and nucleosome reassembly on Chd1. Genes Dev. 26, 914–919.

Meissner, A. (2010). Epigenetic modifications in pluripotent and differentiated cells. Nat. Biotechnol. 28, 1079–1088.

Minsky, N., Shema, E., Field, Y., Schuster, M., Segal, E., and Oren, M. (2008). Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. Nat. Cell Biol. 10, 483–488.

Noer, A., Lindeman, L.C., and Collas, P. (2009). Histone H3 modifications associated with differentiation and long-term culture of mesenchymal adipose stem cells. Stem Cells Dev. 18, 725–736.

Park, G., Gong, Z., Chen, J., and Kim, J.E. (2010). Characterization of the DOT1L network: implications of diverse roles for DOT1L. Protein J. 29, 213–223.

Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A., and Reinberg, D. (2006). Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 125, 703–717.

Pirngruber, J., Shchebet, A., and Johnsen, S.A. (2009a). Insights into the function of the human P-TEFb component CDK9 in the regulation of chromatin modifications and co-transcriptional mRNA processing. Cell Cycle 8, 3636–3642.

Pirngruber, J., Shchebet, A., Schreiber, L., Shema, E., Minsky, N., Chapman, R.D., Eick, D., Aylon, Y., Oren, M., and Johnsen, S.A. (2009b). CDK9 directs H2B monoubiquitination and controls replication-dependent histone mRNA 3′ end processing. EMBO Rep. 10, 894–900.

Prenzel, T., Begus-Nahrmann, Y., Kramer, F., Hennion, M., Hsu, C., Gorsler, T., Hintermair, C., Eick, D., Kremmer, E., Simons, M., et al. (2011). Estrogen-dependent gene transcription in human breast cancer cells relies upon proteasome-dependent monoubiquitination of histone H2B. Cancer Res. 71, 5739–5753.

Shchebet, A., Karpiuk, O., Kremmer, E., Eick, D., and Johnsen, S.A. (2012). Phosphorylation of cyclin-dependent kinase-9 controls ubiquitin-conjugating enzyme-2A function. Cell Cycle 11, 1–6.

Shema, E., Tirosh, I., Aylon, Y., Huang, J., Ye, C., Moskovits, N., Raver-Shapira, N., Minsky, N., Pirngruber, J., Tarcic, G., et al. (2008). The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. Genes Dev. 22, 2664–2676.

Simone, C., Stiegler, P., Bagella, L., Pucci, B., Bellan, C., De Falco, G., De Luca, A., Guanti, G., Puri, P.L., and Giordano, A. (2002). Activation of MyoD-dependent transcription by cdk9/cyclin T2. Oncogene 21, 4137–4148.

Simonsen, J.L., Rosada, C., Serakinci, N., Justesen, J., Stenderup, K., Rattan, S.I., Jensen, T.G., and Kassem, M. (2002). Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat. Biotechnol. 20, 592–596.

Sims, R.J., III, Millhouse, S., Chen, C.F., Lewis, B.A., Erdjument-Bromage, H., Tempst, P., Manley, J.L., and Reinberg, D. (2007). Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. Mol. Cell 28, 665–676.

Vethantham, V., Yang, Y., Bowman, C., Asp, P., Lee, J.H., Skalnik, D.G., and Dynlacht, B.D. (2012). Dynamic loss of H2B ubiquitylation without corresponding changes in H3K4 tri-methylation during myogenic differentiation. Mol. Cell Biol. 32, 1044–1055.

Yu, W., Wang, Y., Shaw, C.A., Qin, X.F., and Rice, A.P. (2006). Induction of the HIV-1 Tat co-factor cyclin T1 during monocyte differentiation is required for the regulated expression of a large portion of cellular mRNAs. Retrovirology 3, 32. 10.1186/1742-4690-3-32.

Zhang, F., and Yu, X. (2011). WAC, a functional partner of RNF20/40, regulates histone H2B ubiquitination and gene transcription. Mol. Cell 41, 384–397.

Zhang, F., Barboric, M., Blackwell, T.K., and Peterlin, B.M. (2003). A model of repression: CTD analogs and PIE-1 inhibit transcriptional elongation by P-TEFb. Genes Dev. 17, 748–758.

Zhu, B., Zheng, Y., Pham, A.D., Mandal, S.S., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2005). Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol. Cell 20, 601–611.