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Identification of multiple roles for histone acetyltransferase 1 in replication-coupled chromatin assembly

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ABSTRACT

Histone acetyltransferase 1 (Hat1) catalyzes the acetylation of newly synthesized histone H4 at lysines 5 and 12 that accompanies replication-coupled chromatin assembly. The acetylation of newly synthesized H4 occurs in the cytoplasm and the function of this acetylation is typically ascribed to roles in either histone nuclear import or deposition. Using cell lines from Hat1$^{+/+}$ and Hat1$^{-/-}$ mouse embryos, we demonstrate that Hat1 is not required for either histone nuclear import or deposition. We employed quantitative proteomics to characterize Hat1-dependent changes in the composition of nascent chromatin structure. Among the proteins depleted from nascent chromatin isolated from Hat1$^{-/-}$ cells are several bromodomain-containing proteins, including Brg1, Baz1A and Brd3. Analysis of the binding specificity of their bromodomains suggests that Hat1-dependent acetylation of H4 is directly involved in their recruitment. Hat1$^{-/-}$ nascent chromatin is enriched for topoisomerase 2α and 2β. The enrichment of topoisomerase 2 is functionally relevant as Hat1$^{-/-}$ cells are hyper-sensitive to topoisomerase 2 inhibition suggesting that Hat1 is required for proper chromatin topology. In addition, our results indicate that Hat1 is transiently recruited to sites of chromatin assembly, dissociating prior to the maturation of chromatin structure.

INTRODUCTION

DNA packaging into chromatin structure is a highly regulated step in DNA replication, which guarantees not only a high degree of compaction of the DNA but also the proper transmission of epigenetic information to daughter cells (1–4). The packaging of replicating DNA into the correct chromatin structure is critically dependent on the proper processing, transport and assembly of newly synthesized histones.

The replication-coupled assembly of newly synthesized histones into chromatin is typically described as starting in the cytoplasm, where the histones are synthesized in the large quantities necessary to support genome duplication. The first processing step appears to be the methylation of new histone H3 lysine 9, which occurs during translation (5–7). The histones then oligomerize to form stable H3/H4 and H2A/H2B dimers (8,9). Once dimerized, a second processing step occurs with the acetylation of lysines 5 and 12 on the NH_2-terminal tail of histone H4 by the Hat1 complex, which contains the histone acetyltransferase Hat1 and the histone chaperone Rbap46 (Hat2 in yeast) (10–14). It has been proposed that the modified H3/H4 dimers are then transferred to the histone chaperone Asf1 through the direct association of Asf1 with the Hat1/Rbap46/H3/H4 complex. The Asf1/H3/H4 complex can then associate with specific importins/karyopherins to facilitate the nuclear import of the H3/H4 dimers (8,9,15–18). Once in the nucleus, additional processing occurs with the acetylation of H3 lysine 56 and additional lysine residues in the H3 NH_2-terminal tail (15,19–30). The modified H3/H4 complexes are then transferred to the CAF-1 complex (Chromatin Assembly Factor 1), which facilitates the deposition of H3/H4 tetramers near replication forks through a physical interaction with PCNA (31–39). The new H2A/H2B dimers, which do not appear to be post-translationally modified, are likely to associate with distinct histone chaperones in the cytoplasm, such as Nap-1, which facilitate their nuclear import and assembly onto the nascent H3/H4 tetramers to form nucleosomes (17,40). The nascent nucleosomes do not gain the requisite level of stability until they undergo a process of maturation. During this chromatin maturation a number of events occur, including the removal of the acetylation marks acquired by H3 and H4 during the assembly process and the association of linker histones (41–45).

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The pattern of di-acetylation on newly synthesized histone H4 is highly conserved among eukaryotes (46–51). This evolutionary conservation and the strong temporal correlation between the acetylation of newly synthesized H4 molecules near the start of the chromatin assembly process and their deacetylation at the completion of assembly during maturation suggests that this modification plays an important role in replication-coupled chromatin assembly (47,52). Potential functions for the di-acetylation of newly synthesized H4 include facilitating nuclear import, regulating interactions with histone chaperones or modulating the wrapping of DNA on the H3/H4 tetramer during deposition. However, several studies have shown that, at least in yeast, the absence of acetylation on H4 lysines 5 and 12 has no effect on chromatin assembly or cell proliferation (53–56). In addition, loss of either the NH_2-terminal tail of H3 or H4 has no effect on nucleosome deposition mediated by CAF-1 (57), leaving the biological relevance of this modification unclear.

Hat1/Rbap46 complexes are also widely conserved in eukaryotes. However, despite this evolutionary conservation, the biological relevance of this enzyme remains to be elucidated. Deletion of *HAT1* in *Saccharomyces cerevisiae* has no effect on cell proliferation or global chromatin assembly (10,11,58). However, biochemical and molecular genetic evidence suggests that HAT1 may function in histone dynamics. First, deletion of *HAT1* in yeast results in defects in telomeric silencing and DNA double strand break repair if the *HAT1* deletion is combined with lysine to arginine mutations in the histone H3 NH_2-terminal tail (59,60). Second, the HAT1/H3 tail mutants have delayed assembly of chromatin structure following repair of a double strand break (61). Interestingly, subsequent studies, in a variety of systems from fungi to mammalian cells, have shown that Hat1 mutations on their own result in similar phenotypes (13,62–66). Further support for an important role of the Hat1/Rbap46 complex in histone dynamics comes from numerous proteomic studies that have shown that Hat1 and Rbap46 are primary components of soluble H3/H4 complexes (8,9,18,67–73).

Deletion of HAT1 in mice leads to neonatal lethality due to developmental lung defects. In addition, primary mouse embryonic fibroblasts (MEFs) obtained from $Hat1^{-/-}$ mice display a significant growth defect relative to their wild type counterparts, and exhibit increased DNA damage sensitivity and genome instability. Importantly, Hat1 was shown to be required for the post-translational modification of newly synthesized H4. Consistent with this observation, Hat1 was found to be essential for the incorporation of K5 and K12 di-acetylated H4 during replication coupled chromatin assembly. Surprisingly, Hat1 is also necessary for the replication-dependent incorporation of newly synthesized H3 acetylated on lysines 9 and 27 (13).

$Hat1^{+/+}$ and $Hat1^{-/-}$ MEF cell lines provide a model system to answer the long-standing question of the role Hat1 and the acetylation of newly synthesized histones play in mammalian replication-coupled chromatin assembly. We demonstrate that Hat1 is not required for either nuclear import or deposition of core histones. Using quantitative proteomics, we identify differences in nascent chromatin between $Hat1^{+/+}$ and $Hat1^{-/-}$ MEFs. In the absence of Hat1, there is a decrease in the abundance of a subset of bromodomain-containing proteins, including Brg1, Baz1a and Brd3. Analysis of the binding of these proteins to modified peptide arrays is consistent with a direct role of new histone acetylation in the recruitment of these proteins to nascent chromatin. In addition, loss of Hat1 results in an enrichment of topoisomerase 2 and DNA helicases suggesting that Hat1 plays a role in ensuring proper chromatin topology during assembly. Intriguingly, we also demonstrate that Hat1 is localized to newly replicated DNA with kinetics similar to H4 lysine 5 and 12 acetylation and is evicted from chromatin during the chromatin maturation process, indicating that Hat1 may play a direct role in orchestrating the assembly and maturation of chromatin structure.

MATERIALS AND METHODS

MEF cell culture

E14.5 embryos were dissected from uterus; head, limbs and internal organs were removed. Tissue was disaggregated using an 18-gauge syringe and brought to single cell suspension with trypsin incubation at 37°C. Cells were then plated onto 100 mm tissue culture plates, passaged upon confluency and maintained in Dulbecco's modified Eagle medium (DMEM-Sigma) with 10% fetal bovine serum (FBS-Gibco) and 1X Pen/Strep antibiotics (Sigma). SV40 T immortalized MEFs (iMEFs) were derived from primary $Hat1^{+/+}$ and $Hat1^{-/-}$ embryonic day 13.5 embryos. To establish iMEFs, early passage cells were transformed with SV-40 T antigen containing plasmid pBSSVD2005 (ADDGENE, Cambridge, MA). Early passage cells were seeded at 25% confluency in six-well plates and transfected with 2 ug of expression vector using Fugene reagent (Roche). Cells were harvested and seeded into 100 mm dishes after 48 h of transfection. The cells were split at 1 in 10 dilutions until passage 5.

For the SILAC experiments, cells were grown in SILAC DMEM media (ThermoFisher Scientific) and supplemented with 10% dialyzed fetal bovine serum (ThermoFisher Scientific), antibiotics and isotopically labeled lysine and arginine (Cambridge Isotope laboratories). $Hat1^{-/-}$ cells were grown under these conditions for at least four passages to guarantee the full incorporation of the labeled amino acids.

Immunofluorescence

iMEFS were seeded on six-well plates containing coverslips. Cells were fixed with 4% paraformaldehyde, blocked with 5% BSA and primary antibodies against histone H4 (Abcam) were incubated overnight, washed and replaced with goat-anti-rabbit secondary antibody. Cells were mounted in Vectashield mounting media containing DAPI and imaged using a wide field fluorescence microscope.

Isolation of proteins on nascent DNA (iPOND)

8×10^7 iMEFs were incubated with 10 μM EdU (Invitrogen) for different time periods. For thymidine chase experiments, EdU labeled cells were washed once with pre-equilibrated (temperature, pH and thymidine) medium and

then incubated with 10 μM thymidine for various times. After labeling and/or pulse-chase, cells were cross-linked with 1% formaldehyde/PBS for 20 min, quenched with 1.25 M glycine and scraped off the plates and collected. After washing three times with PBS, the cells were permeabilzed by resuspension in 0.25% Triton-X100/PBS. Cells were washed with 0.5% BSA/PBS; and incubated with either click reaction buffer (10 mM biotin azide, 10 mM sodium ascorbate, 2 mM $CuSO_4$ in PBS) or control buffer (same as reaction buffer but DMSO added instead of biotin azide) 1 h at room temperature, protected from light. After incubation, cells were again washed with 0.5% BSA/PBS. Cells were then lysed with lysis buffer (1% SDS, 50 mM Tris pH 8.0, 1 μg/ml Leupeptin, 1 μg/ml aprotinin). Samples were then sonicated using the Bioruptor (Diagenode) for 30 s on and 60 s off per cycle for 12 cycles. Samples were then spun down and supernatant was filtered through 90 micron nylon mesh (Small Parts) and diluted with PBS containing protease inhibitors. An aliquot of the lysate was kept as input, the rest was incubated with prewashed Streptavidin-agarose beads (Novagen) for 18 h at 4°C. The beads were then washed once with lysis buffer, once with 1 M NaCl, and twice with lysis buffer. Beads were boiled with SDS dye for 25 min at 95°C. Proteins were resolved by SDS-PAGE and detected by western blot using antibodies against HAT1, H3, H4, H2A and H2B (Abcam).

In-gel digestion of iPOND samples for mass spectometry

Boiled samples were loaded into a TGX 4–15% precast gel. The gel was run until the dye front was ∼2 cm below the bottom of the loading well. Following fixation, the gel was stained with Coomassie (Bio-Rad). Disulfide bonds were reduced with DTT and alkylated with iodoacetamide prior to digestion with 600 ng trypsin (Promega). Peptides were extracted from the gel matrix, dried under vacuum and resuspended in loading buffer (2% acetonitrile, 0.1% formic acid).

LC–MS/MS

Each biological replicate was analyzed by LC–MS/MS in technical duplicate. Peptides (1000 ng/sample) were loaded on a PepMap100 C18 microcolumn (5 μm, 100 Å, 0.3 × 50 mm) and desalted for 6 min with 0.1% TFA in 2% acetonitrile. Mobile phase solvents were buffer A: 0.1% formic acid in water and buffer B: 0.1% formic acid in acetonitrile. Separation was performed using a Dionex UltiMate 3000 RSLCnano HPLC system interfaced with an EASY-Spray PepMap C18 column (3 μm, 100 Å, 0.75 × 150 mm) operated at 275°C and a spray voltage of 1.7 kV. Peptides were eluted along a linear gradient (5–28% buffer B) at a flow rate of 300 nl/min over 250 min followed by a column wash/equilibration step.

MS/MS data were collected on an Orbitrap Fusion operated in top-speed mode with a cycle time of 3 s. Precursor ions were isolated in the quadrupole (width 1.6 m/z) and detected in the Orbitrap mass analyzer with 120K resolution. Following CID fragmentation at 35% collision energy, fragment ions were detected in the linear ion trap. AGC targets were 2E5 ions or 50 ms max inject time for MS1 scans and 3E3 ions or 250 ms max inject time for MS2 scans, respectively. Ions within a ±10 ppm window were excluded from repeat fragmentation for 60 s.

Data analysis

RAW data were uploaded to MaxQuant (v1.5.3.17) (74) and searched against a murine UniProt Swiss-Prot database (downloaded 20 November 2015; 16 741 entries) using the Andromeda search engine. Three biological replicates were analyzed as separate experiments, each with two combined technical analyses. Oxidation of M and acetylation of protein NH_2-termini were included as variable modifications and carbamidomethylation of C was included as a fixed modification. Up to two missed cleavages were allowed and the peptide minimum length was set to seven residues with up to three amino acids labeled with Arg10/Lys8. Enzyme specificity was set for trypsin/P. The peptide mass tolerance was fixed at 20 ppm with a fragment mass tolerance of 0.5 Da. Protein and PSM FDRs were set at 0.05. The match between runs feature was enabled with default settings. Quantitation was performed for proteins with at least two unique or razor peptides and any of the variable/fixed modifications described above.

Heavy/light SILAC ratios were calculated in Perseus using intensity values from the MaxQuant proteinGroups output file. Proteins with three valid SILAC ratios (i.e. proteins quantified on both channels in at least one of two technical replicates across all three biological replicates) were kept for further analysis. A two-tailed t-test was performed in Excel followed by the calculation of a multi-test corrected q-value. Data were visualized in Excel or R.

Micrococcal nuclease digestion assays

1×10^7 $Hat1^{+/+}$ and $Hat1^{-/-}$ cells were collected, washed with cold PBS and incubated in lysis buffer (300 mM HEPES pH 7.5, 60 mM KCl, 300 mM sucrose, 5 mM K_2HPO_4, 5 mM $MgCl_2$, 2 mM EDTA, and 0.5% Triton X-100) for 5 min on ice. Cells were lysed with 7 strokes in a Dounce homogenizer (type B). Nuclei were isolated by centrifugation at 120 × g for 10 min. Nuclei were washed with 1 ml MNase digestion buffer (10 mM Tris pH7.5, 15 mM NaCl, 1 mM $CaCl_2$, 60 mM KCl, and 0.2 mM phenyl methyl sulfonate fluoride), resuspended in 1 ml MNase digestion buffer, and digested with 0.2 U/ml (Sigma units) of MNase at 37°C for various lengths of time. Reactions were stopped with 10 mM EDTA followed by incubation with 0.1 mg/ml proteinase K at 37°C for 5 min. DNA was then isolated using phenol extraction and ethanol precipitation and resolved by electrophoresis in a 1% agarose gel.

Histone peptide binding assay

Biotinylated histone peptides (Epicypher) were diluted to 1μM in 0.1% (v/v) triton-X100 in PBS and incubated with streptavidin beads (Novagen) for 3 h at room temperature. After three washes with binding buffer (50 mM Tris–HCl, pH 7.5, 250 mM NaCl, 0.05% (v/v) NP-40) peptide-bound beads were incubated with 100 nM GST-tagged recombinant proteins (Baz1a and Brg1, Epicypher; Brd3, Abcam) 3

h at room temperature. After binding, beads were washed three times with binding buffer and subsequently boiled with 2× SDS sample buffer and analyzed by western blotting.

RESULTS

Based on the evolutionarily conserved nature of the enzyme and the di-acetylation of newly synthesized histone H4 that it catalyzes, it has long been hypothesized that Hat1 plays a fundamental role in the deposition of histones during replication-coupled chromatin assembly (52,75–77). We have used Hat1$^{+/+}$ and Hat1$^{-/-}$ MEF cell lines to analyze what, if any, aspects of replication-coupled chromatin assembly are defective in the absence of Hat1.

Hat1 is not required for nuclear import or core histone deposition

Recent reports have suggested that the acetylation of newly synthesized histones is necessary for their proper nuclear import (9,70,78). As defects in histone import would be manifested as an increase in cytosolic histones or a decrease in nuclear histone content, anti-H4 antibodies were used to detect this histone by immunofluorescence; surprisingly there was no detectable increase in cytosolic histone H4 and no significant decrease in nuclear histone H4 in Hat1$^{-/-}$ MEFs (Figure 1A and B).

Once in the nucleus, the H3/H4 complexes must be targeted to sites of DNA replication and assembled onto the replicating DNA as a tetramer. Acetylation on the newly synthesized histones has been proposed to influence their binding to histone chaperones or to modulate the wrapping of the negatively charged DNA onto the positively charged histones (66,79). To determine whether Hat1 is required for core histone deposition, we used iPOND (Isolation of Proteins on Nascent DNA) to analyze the association of the core histones with newly replicating DNA. iPOND uses the thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) to label DNA that is being actively replicated; after crosslinking protein–DNA complexes, the EdU that has been incorporated into the newly replicated DNA can be bound to biotin using click chemistry, allowing the purification of the nascent DNA using streptavidin agarose beads. Specific proteins can be detected using western blot analysis of the fractions eluted from the beads (80).

Immortalized Hat1$^{+/+}$ and Hat1$^{-/-}$ MEFs were labeled with EdU for increasing lengths of time to monitor the kinetics of core histone deposition on newly replicated DNA (Figure 1C). As has been reported previously, the acetylation of histone H4 assembled onto the newly replicated DNA is almost entirely dependent on Hat1 (Figure 1C) (13). PCNA, a component of the replisome, is detectable on newly replicated DNA at the 10 min time point and its levels quickly reach an equilibrium as the addition of new replisomes is balanced by the dissociation of PCNA following passage of the replication fork. In contrast, the levels of histone proteins on the replicating DNA increase with increasing times of labeling, as the histones are stably associated with the DNA. The rate at which H3 and H4 associate with newly replicated DNA is not decreased in Hat1$^{-/-}$ MEFs and, in fact, may be somewhat more robust in the absence of Hat1. In addition, the deposition of H2A and H2B was not diminished by the loss of Hat1. These data suggest that Hat1-dependent acetylation of newly synthesized histones is not required for either histone import or deposition.

Hat1 influences the nascent chromatin proteome

We combined iPOND with SILAC (Stable Isotope Labeling of Amino acids in Culture) quantitative mass spectrometry to obtain an unbiased determination of Hat1-dependent changes in nascent chromatin protein composition. Immortalized Hat1$^{+/+}$ and Hat1$^{-/-}$ cells were grown in normal media or media containing isotopically labeled lysine and arginine, respectively. The cells were then pulse labeled for 30 min with EdU. Equal numbers of cells were combined and processed as described above for iPOND. Following streptavidin purification of EdU labeled DNA, proteins associated with nascent chromatin were subjected to in-gel tryptic digestion and the resulting peptides analyzed by mass spectrometry. Changes in the abundance of a protein on nascent chromatin between the Hat1$^{+/+}$ and Hat1$^{-/-}$ cells were then quantified by determining the ratios of peptides containing isotopically labeled amino acids to normal amino acids (Figure 2A).

Three different clones each of Hat1$^{+/+}$ and Hat1$^{-/-}$ cells were analyzed in technical duplicates. The raw data obtained were filtered with high stringency and only proteins with valid SILAC ratios in all of the biological replicates were considered in the initial analysis. The biological replicates were highly reproducible and the $\log_2$ SILAC ratios clustered near 0 (Supplementary Figure S1). The proteomic analysis identified a total of 474 proteins present on nascent chromatin (Supplementary Table S1, note that ribosomal proteins were not included in further analyses). Gene Ontology analysis of the 474 proteins indicated that the pathways that were the most highly enriched were DNA replication and chromatin organization suggesting that we had successfully isolated nascent chromatin (Figure 2B). Interestingly, proteins involved in the regulation of translation, mRNA splicing and DNA repair were also highly enriched on nascent chromatin.

There have been four recent reports of quantitative proteomic analyses of nascent chromatin (81–84). These proteomic studies of nascent chromatin use different methods of nascent DNA labeling, label replicating DNA for different lengths of time and use different filters for the classification of proteins as a constituent of nascent chromatin. Despite these differences, comparison of our list of nascent chromatin proteins with the lists generated in the published studies shows significant overlap with each of these studies (Supplementary Figure S2). Indeed, overall, the level of overlap was greater than the overlap seen in pairwise combinations of the previously published studies (Supplementary Figure S2).

Figure 3A displays an overview of the 474 proteins identified on nascent chromatin sorted by their change in abundance in the Hat1$^{-/-}$ cells with the most highly enriched proteins at the top and the most highly depleted at the bottom. In addition, each of the proteins is color-coded based on a manual sorting of each protein into one of the indi-

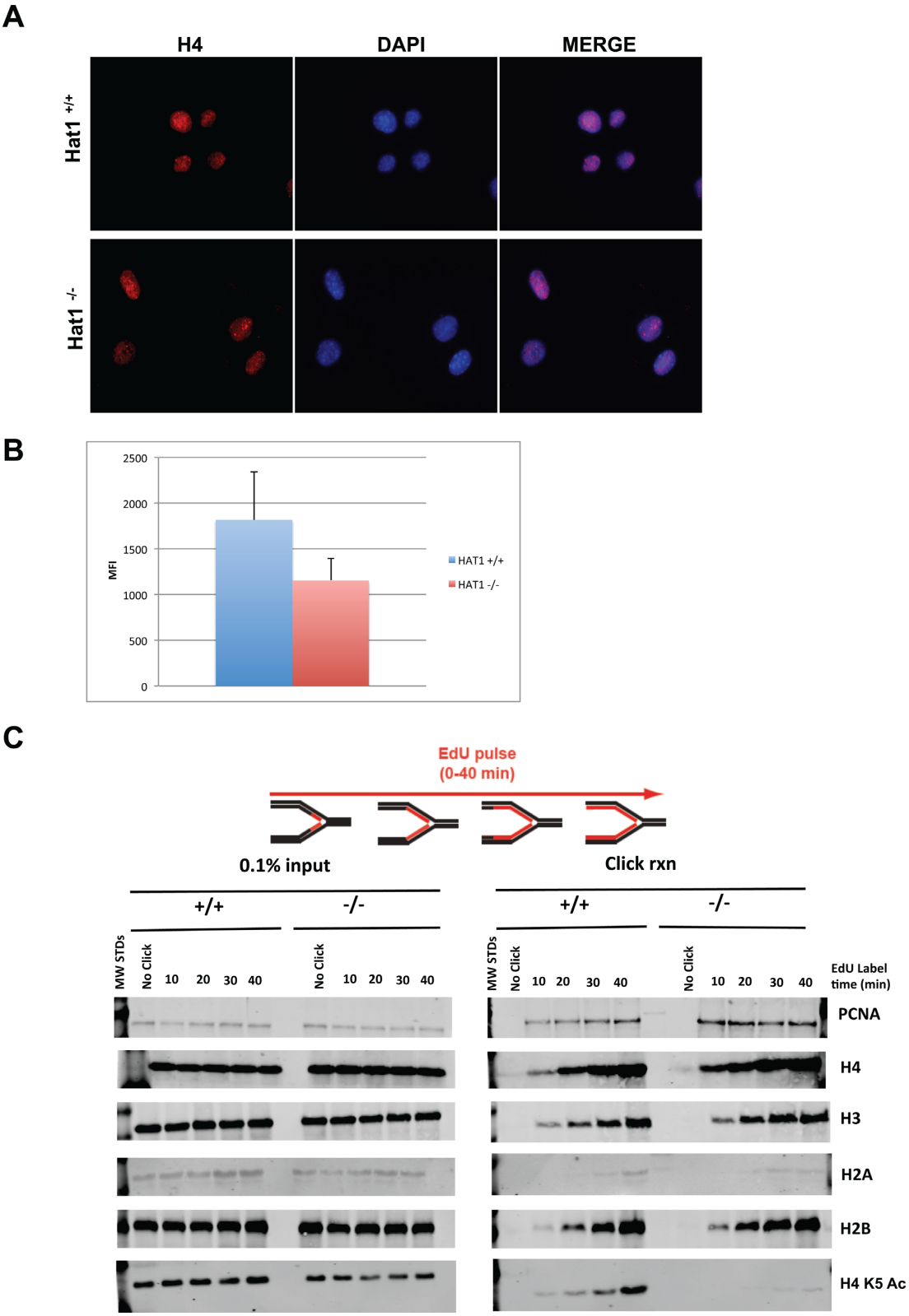
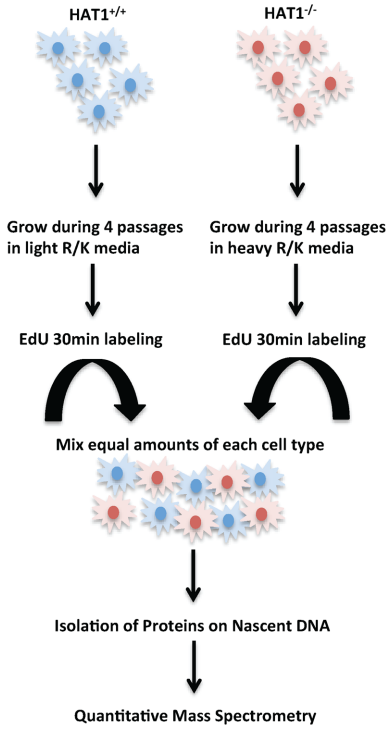


Figure 1. Histone Acetyltransferase 1 is not essential for nuclear import or histone deposition. (**A**) Hat1$^{+/+}$ and Hat1$^{-/-}$ immortalized MEFs were stained with α-H4 antibody and DNA was stained with DAPI. (**B**) Quantification of the mean nuclear fluorescence intensity in cells of the indicated genotype stained with α-H4 antibody. (**C**) Hat1$^{+/+}$ and Hat1$^{-/-}$ cells were pulse labeled with EdU for the indicated time points. Samples were isolated and proteins associated with newly replicated DNA were resolved on SDS-PAGE following iPOND. Western blots were probed with the antibody indicated on the right. No Click control indicates negative control samples in the absence of biotin addition.

A



B

PROCESS	P-VALUE
DNA REPLICATION	4.78×10^{-20}
CHROMATIN ORGANIZATION	1.03×10^{-17}
REGULATION OF TRANSLATION	1.88×10^{-17}
mRNA SPLICING	1.34×10^{-14}
DNA REPAIR	3.76×10^{-11}

Figure 2. Isolation and proteomic analysis of nascent chromatin. (**A**) Schematic diagram of the IPOND-SILAC MS approach. See Materials and Methods for details. (**B**) Biological processes significantly enriched in nascent chromatin. Gene ontology analysis was performed using PANTHER pathway classification system.

cated functional categories. Figure 3B displays the same list in volcano plot format to indicate the statistical significance of the observed changes in abundance based on the consistency observed in the replicate samples. It is clear from these analyses that the majority of the proteins on nascent chromatin are unaffected by the loss of Hat1 and displayed <50% depletion or enrichment in the Hat1$^{-/-}$ cells. Importantly, proteins involved in DNA replication are largely unchanged in the absence of Hat1 indicating that this enzyme does not directly impact DNA replication.

Of the 474 proteins detected on nascent chromatin, 28 (6%) are depleted by more than 50% on the Hat1$^{-/-}$ chromatin. Strikingly, of these, 25 have no known chromatin-related function (Figure 3C). For example, the most highly depleted proteins in the Hat1$^{-/-}$ nascent chromatin were CEP162, mouse centrosomal protein of 162 kDa, which plays a role in the formation of cilia with knockdown this protein causing body axis and other embryonic defects in zebrafish and RNF213, an E3 ubiquitin ligase that is a susceptibility gene for Moyamoya disease (85–88). Consistent with the role of Hat1 in the acetylation of newly synthesized H4 during chromatin assembly, we also observed a significant decrease in peptides from the NH_2-terminal tail of H4 that contain acetylation on lysines 5 or 12 (Supplementary Table S2). We were not able to directly observe H4 peptides that contain the diacetylation of lysines 5 and 12 as these peptides are cleaved by trypsin after the unmodified lysine at position 8.

Three proteins depleted by >50% in the Hat1$^{-/-}$ nascent chromatin have known chromatin functions or associations (Figure 3D). The most highly depleted chromatin-related protein is Brg1, which is an ATP-dependent chromatin-remodeling enzyme (89). The second most depleted chromatin-related protein is 14-3-3 ϵ. 14-3-3 ϵ is a member of a family of proteins that have been shown to interact with histones in a modification-dependent manner and three other 14-3-3 family members also show moderate levels of depletion from the Hat1$^{-/-}$ nascent chromatin (90–94). MTA1 is a component of the chromatin modifying and remodeling complex NuRD. Other components of the NuRD complex, such as MBD3, HDAC1, Rbap46, Rbap48 and CHD4, are also detected on nascent chromatin but their levels are not significantly altered by the loss of Hat1 (95,96). The lone exception is Rbap46 and that will be discussed in more detail below.

Hat1 promotes recruitment of bromodomain-containing proteins to nascent chromatin

The decreased abundance of Brg1 on Hat1$^{-/-}$ nascent chromatin may be linked to the presence of bromodomains in this protein. Bromodomains are motifs that have been demonstrated to recognize and bind to acetylated lysine residues (97–99). In addition to Brg1, two other bromodomain-containing proteins, Brd3 and Baz1a, were identified on nascent chromatin and were depleted from the Hat1$^{-/-}$ chromatin by 25–30% (Figure 4A). Interestingly, both Brg1 and Baz1a have been reported to have a role in DNA replication and chromatin assembly with Baz1a being a component of the ACF complex that generates regularly spaced nucleosomes during chromatin assembly (100,101). In addition, Brd3 has been shown to associate with chromatin in vivo that is enriched in histone H4 lysine 5 and lysine 12 acetylation (102). To test the hypothesis that the association of these bromodomain-containing proteins with nascent chromatin is mediated by the Hat1-dependent acetylation of newly synthesized histones, we analyzed the binding of these proteins to a series of synthetic biotin labeled peptides derived from the NH_2-terminal tail of histone H4. In addition to the unmodified H4 tail, we also tested peptides that contain mono-acetylation at either lysine 5, 8 12 or 16 and peptides that contained diacetylation at either lysines 5 and 12 or lysines 8 and 16. Recombinant GST-bromodomain protein fusions were incubated with the peptides and then precipitated using streptavidin agarose beads. Brg1 displayed minimal binding to the unmodified H4 NH_2-terminal tail peptide. However, binding to the H4 tail was significantly enhanced by the pres-

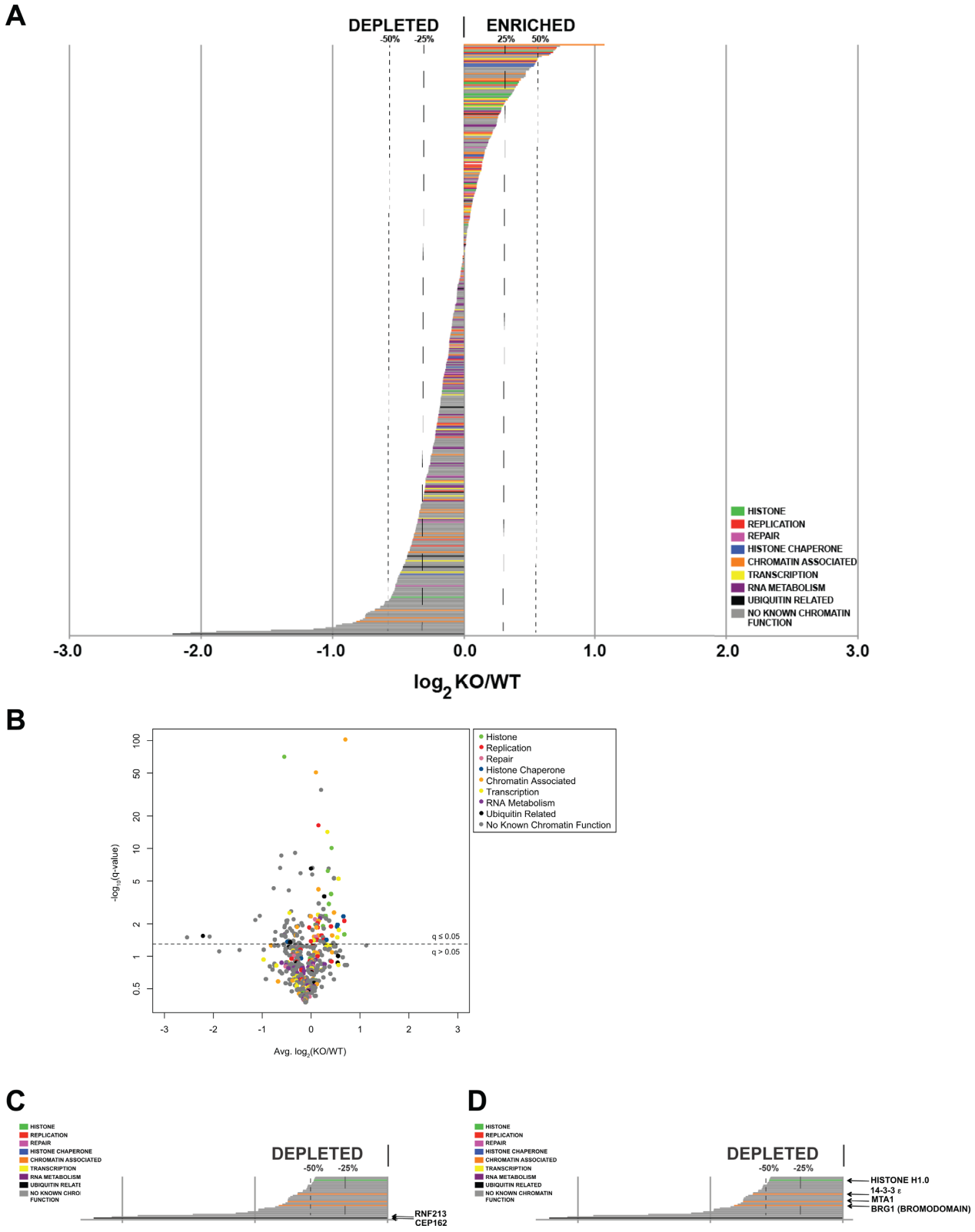
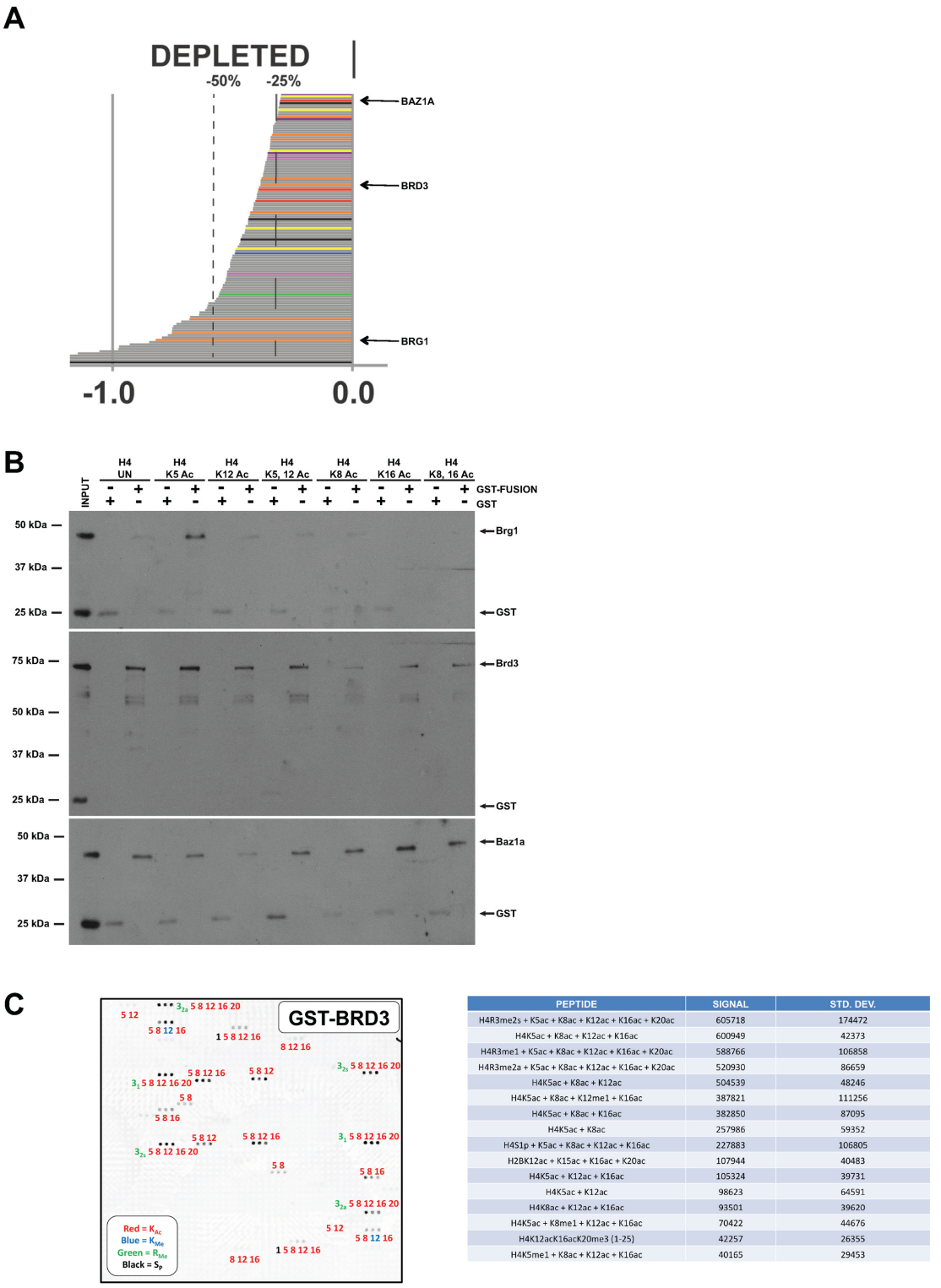


Figure 3. Histone acetyltransferase 1 influences nascent chromatin proteomics. (**A**) General overview of the 474 proteins detected by mass spec in all three Hat$^{+/+}$ and Hat1$^{-/-}$ samples. Length of the bar for each proteins indicates the degree of enrichment (to the right) or depletion (to the left) observed for each protein in Hat1$^{-/-}$ nascent chromatin relative to Hat1$^{+/+}$ nascent chromatin. Dashed lines indicate the threshold for proteins that were different by 25% or more; dotted line indicates the threshold for those proteins that were different by 50% or more. Colors indicate the different biological process to which each of the proteins is associated. (**B**) Volcano plot of the 474 proteins identified in in the comparison of Hat1$^{+/+}$ and Hat1$^{-/-}$ nascent chromatin. Dotted line indicates a q value of 0.05. (**C** and **D**) Detailed view of the proteins depleted in absence of Hat1.



PEPTIDE	SIGNAL	STD. DEV.
H4R3me2s + K5ac + K8ac + K12ac + K16ac + K20ac	605718	174472
H4K5ac + K8ac + K12ac + K16ac	600949	42373
H4R3me1 + K5ac + K8ac + K12ac + K16ac + K20ac	588766	106858
H4R3me2a + K5ac + K8ac + K12ac + K16ac + K20ac	520930	86659
H4K5ac + K8ac + K12ac	504539	48246
H4K5ac + K8ac + K12me1 + K16ac	387821	111256
H4K5ac + K8ac + K16ac	382850	87095
H4K5ac + K8ac	257986	59352
H4S1p + K5ac + K8ac + K12ac + K16ac	227883	106805
H2BK12ac + K15ac + K16ac + K20ac	107944	40483
H4K5ac + K12ac + K16ac	105324	39731
H4K5ac + K12ac	98623	64591
H4K8ac + K12ac + K16ac	93501	39620
H4K5ac + K8me1 + K12ac + K16ac	70422	44676
H4K12acK16acK20me3 (1-25)	42257	26355
H4K5me1 + K8ac + K12ac + K16ac	40165	29453

Figure 4. Histone acetyltransferase 1 influences the recruitment of bromodomain proteins to nascent chromatin. (**A**) Detailed view of proteins depleted in absence of Hat1; arrows point to the chromatin modifiers containing bromodomains that were found depleted in absence of Hat1. (**B**) Peptides encoding the first 23 amino acids of histone H4 containing the modifications indicated across the top were incubated with recombinant Brg1 (top), Brd3 (middle) and Baz1a GST fusions. Each peptide was also separately incubated with a control GST protein. Protein–histone peptide complexes were then precipitated with streptavidin agarose beads. Proteins bound to the beads were then resolved by SDS-PAGE and visualized with anti-GST antibodies. INPUT lane contains the relative quantity of the GST control and protein GST fusion protein that were individually added to each reaction. (**C**) Histone peptide array (Epititan, Epicypher) was incubated with recombinant Brd3-GST fusion protein. Peptides bound by Brd3-GST were visualized and quantitated per manufactures instructions. The left panel is an image of the array probed with Brd3-GST. Peptides bound by Brd3 are labeld according to the legend (note that the subscript s or a refers to the symmetric or asymmetric methylation of the indicated arginine residue. The right panel table lists the modified peptides that were bound Brd3-GST.

ence of lysine 5 acetylation (Figure 4B). Brd3, containing bromodomains 1 and 2, interacted with the unmodified H4 tail peptide and this binding was enhanced most strongly by acetylation at lysine 5 and also, to a lesser degree, acetylation at lysine 12 and di-acetylation at lysines 5 and 12 (Figure 4B). Significant binding of Baz1a was only detected in the presence of acetylation on lysine 16 (Figure 4B).

To determine the binding specificity of these bromodomain-containing proteins more comprehensively, the binding specificity of Brg1, Brd3 and Baz1a was analyzed using modified histone peptide microarrays (Epititan, Epicypher). The modified histone peptide array contains 272 peptides that represent a variety of modified forms of the NH_2-terminal tails of all four core histones. Recombinant Brd3-, Brg1- and Baz1a-GST fusion proteins were incubated with the modified histone peptide array and binding visualized using an anti-GST antibody. Binding of Brg1 and Baz1a to the peptide array was too weak to be detected. Figure 4C shows the peptide binding detected for Brd3. Of the 272 peptides present on the array, binding to only 16 peptides was observed and, of these, 15 were from the H4 tail (one peptide was from the H2B tail). Strikingly, all 15 H4 peptides contained acetylation on lysine 5 and/or lysine 12. Taken together, these data suggest that the Hat1-dependent acetylation of newly synthesized histone H4 on lysines 5 and 12 plays a direct role in recruiting a subset of bromodomain-containing proteins to nascent chromatin.

Hat1 is required for the proper topology of newly assembled chromatin

Nascent chromatin from Hat1$^{-/-}$ cells contains decreased levels of components of several ATP-dependent chromatin-remodeling complexes (Brg1, NuRD and ACF) suggesting that the chromatin assembled in the absence of Hat1 may have altered structure or spacing. Indeed, Hat1$^{-/-}$ nascent chromatin contained increased levels of the core histones. As seen in Figure 5A, changes in the abundances of all the core histones were tightly clustered and displayed increases of 25–30%. A similar level of enrichment on Hat1$^{-/-}$ nascent chromatin was observed for several histone chaperones, including the FACT complex (SSRP1 and SPT16H), ATRX and NPM3. Consistent with an increased density of core histones, comparison of MNase digested chromatin from Hat1$^{+/+}$ and Hat1$^{-/-}$ cells indicated that the Hat1$^{-/-}$ chromatin had a shorter repeat length and displayed a less distinct nucleosome ladder, which is particularly apparent for fragments containing more than four nucleosomes (Figure 5B and C).

Analysis of the proteins most highly enriched in Hat1$^{-/-}$ nascent chromatin also suggests that there are topological defects in chromatin assembled in the absence of Hat1 (Figure 6A). Eleven of the 474 proteins identified on nascent chromatin (2%) were found to be >50% enriched on the Hat1$^{-/-}$ chromatin. These included HELLS and CDCA7. HELLS is a DNA helicase that is specifically required for the formation of heterochromatin. The molecular function of CDCA7 is unknown but mutations in both CCDA7 and HELLS can cause immunodeficiency-centromeric instability-facial anomalies syndrome and they function in a common pathway in this disease.

Topoisomerase 2α and Topoisomerase 2β were also highly enriched on Hat1$^{-/-}$ nascent chromatin. This enrichment is specific for type 2 topoisomerases as Topoisomerase 1 is present at equivalent levels on Hat1$^{+/+}$ and Hat1$^{-/-}$ nascent chromatin (Supplementary Figure S1). Topoisomerase 2 plays an essential role in regulating the topology of the genome and is required for many processes that involve manipulation of the genome (103). Topoisomerase 2 activity was recently linked to chromatin assembly by *in silico* modeling studies. The *in silico* chromatin assembly models often generated disorganized chromatin due to the formation of incorrectly folded nucleosomes and the addition of Topoisomerase 2 activity to the models reversed these defects and restored proper chromatin formation (104).

We hypothesized that assembly of chromatin in the absence of Hat1 resulted in improperly formed nucleosomes and that Topoisomerase 2 activities were then recruited to the nascent chromatin to resolve the topological constraints imposed by the defective nucleosomes. This hypothesis predicts that Hat1$^{-/-}$ cells should be hypersensitive to the inhibition of Topoisomerase 2 activity. Therefore, we treated Hat1$^{+/+}$ and Hat1$^{-/-}$ cells with a Topoisomerase 2 inhibitor. ICRF193 was used, as it does not result in the formation of double strand breaks to which Hat1$^{-/-}$ cells are sensitive (105). As expected, given the essential role of Topoisomerase 2, increasing doses of ICRF193 were lethal to Hat1$^{+/+}$ cells but, at each drug concentration, Hat1$^{-/-}$ cells displayed increased sensitivity (Figure 6B). To determine whether the effect of ICRF193 on Hat1$^{-/-}$ cells was restricted to a specific phase of the cell cycle, cells treated with 3 uM ICRF193 were analyzed by flow cytometry at different time points following treatment. As seen in Figure 6C, after 12 h both Hat1$^{+/+}$ and Hat1$^{-/-}$ cells had largely completed S phase and were primarily in G2/M phase. After 24 hours, some cells from both genotypes successfully pass through mitosis to G1 but a significant fraction become aneuploid due to the inhibition of Topoisomerase 2. By 36 hours, the Hat1$^{+/+}$ cells are predominantly in G2/M while a significant fraction of the Hat1$^{-/-}$ cells remained in either G1 or S phase. Hence, while both the Hat1$^{+/+}$ and Hat1$^{-/-}$ cells can traverse the first round of DNA replication in the presence of a Topoisomerase 2 inhibitor, the Hat1$^{-/-}$ cells displayed defects during the second cell cycle. Hence, loss of Hat1 sensitizes cells to Topoisomerase 2 inhibition.

Hat1 may directly regulate chromatin maturation

The initial bioinformatics analysis of the nascent chromatin quantitative proteomics relied on measuring the change in abundance of proteins that were detected in all three of the Hat1$^{+/+}$ and Hat1$^{-/-}$ samples. This analysis did not take into account proteins that were undetectable in the Hat1$^{-/-}$ nascent chromatin. The proteomic data were reanalyzed to capture proteins whose presence on nascent chromatin was completely dependent on Hat1. Five proteins were identified that were present in at least two of the Hat1$^{+/+}$ nascent chromatin samples and were completely absent from the Hat1$^{-/-}$ nascent chromatin (Figure 7A). Kaiso is a tran-

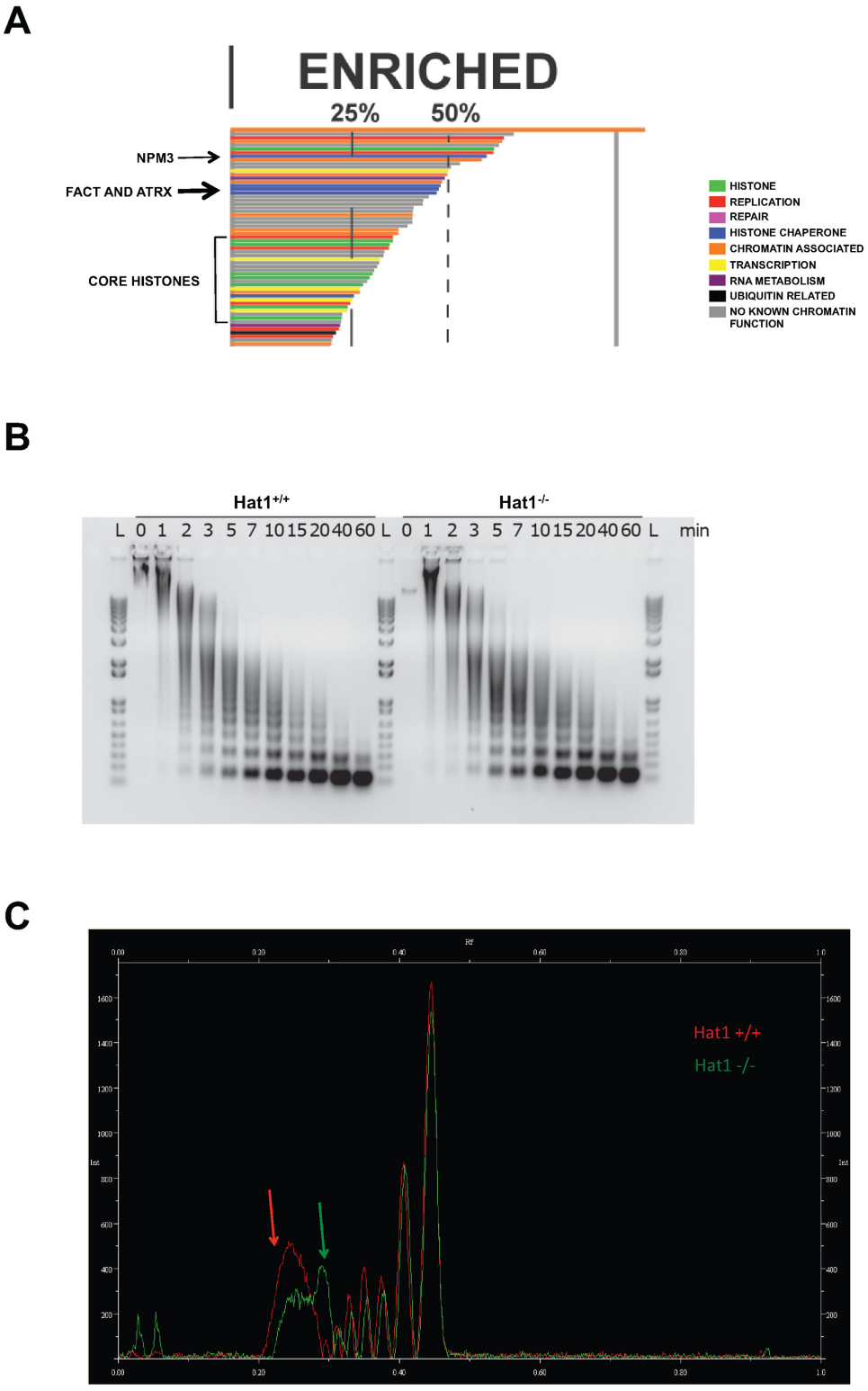


Figure 5. Loss of Hat1 causes an enrichment in histones and histone chaperones. (**A**) Detailed view of the enriched proteins found in Hat1$^{-/-}$ nascent chromatin. Brackets denote core histones (green bars) and arrows denote enriched histones chaperones (blue bars). (**B**) Chromatin from Hat1$^{+/+}$ and Hat1$^{-/-}$ immortalized MEFs was incubated with MNase the for the indicated time points. Digested and deproteinized DNA was separated in 1% agarose gel and stained with Ethidium Bromide. (**C**) Densitometer analysis of the results obtained in (B). Arrows indicate the shift in length between Hat1$^{+/+}$ and Hat1$^{-/-}$ chromatin.

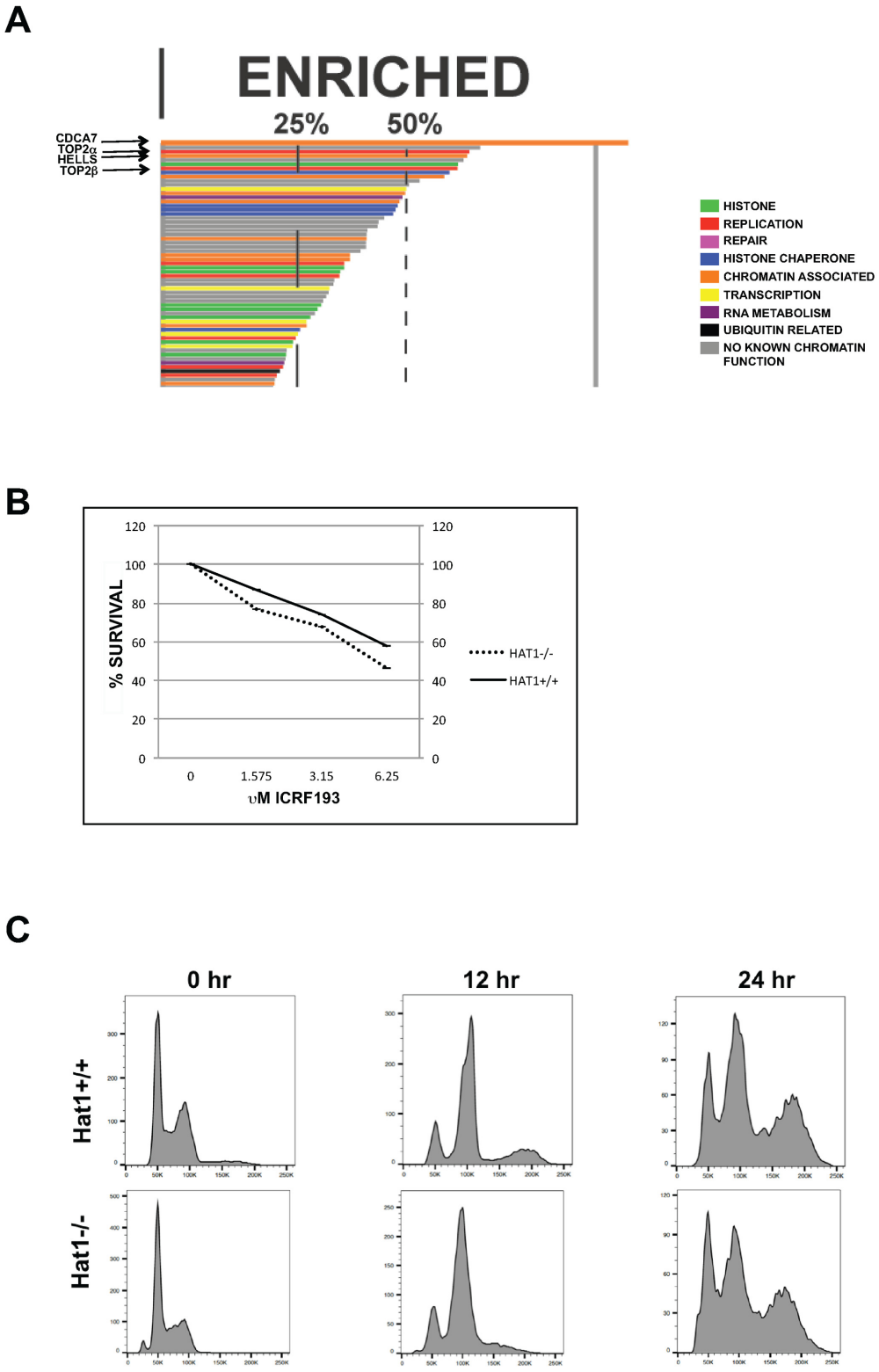


Figure 6. Hat1 loss results in the enrichment of factors regulating chromatin topology. (**A**) Detailed view of enriched proteins in Hat1$^{-/-}$ nascent chromatin, arrows indicate proteins that regulate DNA or chromatin topology enriched by >50%. (**B**) Cells were treated with ICRF193 at the indicated concentrations for 24 h. MTS assays were used to measure cell growth relative to day 0. Results are from three independent experiments ($n = 3$ replicates/experiment). Error bars represent the standard deviation of the three independent experiments. (**C**) Cell cycle analysis by flow cytometry of Hat1$^{+/+}$ and Hat1$^{-/-}$ MEFs treated with vehicle or 3 μM Topoisomerase 2 inhibitor ICRF193 for 12, 24 or 36 h before fixation and staining with propidium iodide.

A

PROTEIN	Hat1^{WT}-1	Hat1^{WT}-2	Hat1^{WT}-3	Hat1^{KO}-1	Hat1^{KO}-2	Hat1^{KO}-3
KAISO	26.02433	25.55429	25.16315	ND	ND	ND
CHIO	25.37166	24.21252	23.78547	ND	ND	ND
PCKGM	18.13981	16.77172	16.89936	ND	ND	ND
GEMIN-5	21.60878	21.7416	ND	ND	ND	ND
HAT1	17.54095	ND	17.71559	ND	ND	ND

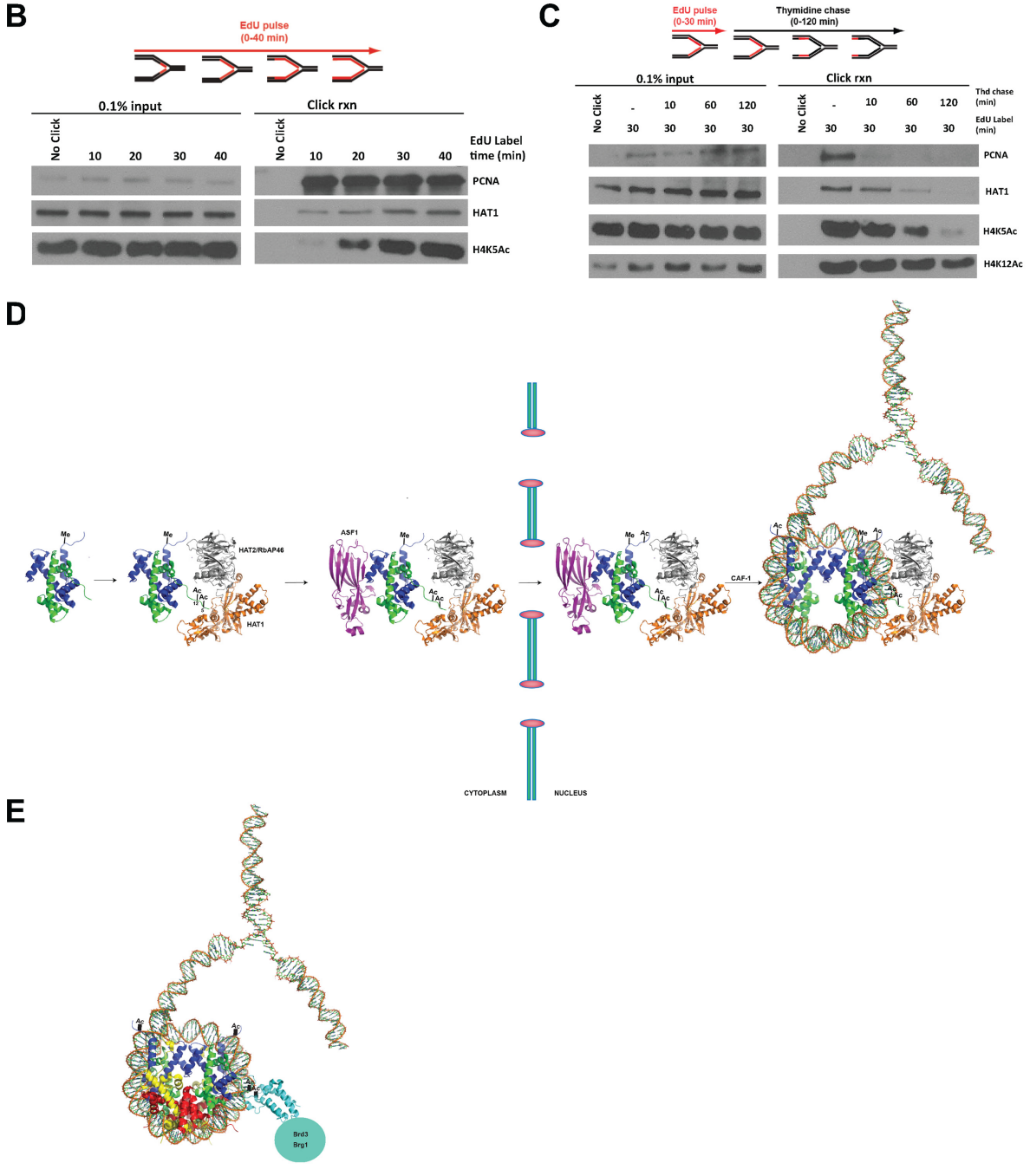


Figure 7. Hat1 transiently associates with nascent chromatin. (**A**) Proteins whose presence on nascent chromatin is completely dependent on Hat1. **B**) HAT1$^{+/+}$ cells were pulse labeled with EdU for the indicated time points. Samples were isolated and proteins associated with newly replicated DNA were resolved on SDS-PAGE following iPOND. Western blots were probed with the antibody indicated on the right. No click control indicates negative control samples. (**C**) Hat1$^{+/+}$ cells were labeled with EdU for 30 min, after several washes cells were chased with thymidine for the indicated time points. Samples were processed as indicated in B. (**D**) Model for the role of Hat1 during replication-coupled chromatin assembly. (**E**) Model indicating the binding of the bromodomains of Brg1 and Brd3 to newly assembled histone H4 in nascent chromatin.

scriptional repressor that can bind methylated DNA and recruit co-repressor complexes (106–109). Chio (chimaerin), PCKGM and Gemin-5 have no know association with chromatin. Interestingly, Hat1 was also detected on two of the $Hat1^{+/+}$ nascent chromatin samples. The presence of Hat1 on nascent chromatin is consistent with a recent report that showed that Hat1 could be immunoprecipitated with BrdU-labelled DNA from *Physarum polycephalum* (70).

To confirm that Hat1 is physically associated with newly assembled chromatin, we performed an iPOND experiment using increasing lengths of EdU labeling. As seen in Figure 7B, Hat1 accumulated on newly replicated DNA with kinetics that were similar to the appearance of histone H4 lysine 5 acetylation. Hat1 has always been found to function in the context of a complex with Rbap46 (Hat2). It is likely that Hat1 is recruited to nascent chromatin as part of a Hat1/Rbap46 complex. Our data show that Rbap46 is the most highly depleted histone chaperone on $Hat1^{-/-}$ nascent chromatin (∼40% depletion). It is likely that Rbap46 is not completely lost from nascent chromatin in the absence of Hat1 due to its presence in other complexes that are present on the nascent chromatin such as the NuRD complex (95,110). A direct demonstration of the association of Rbap46 with newly replicated DNA via iPOND was not feasible as the fixation step of the iPOND procedure resulted in blocking the binding of α-Rbap46 antibodies.

To determine whether Hat1 is a stable component of chromatin or whether it is transiently associated with newly replicated chromatin, we performed iPOND using a pulse chase with EdU and thymidine. From the data in Figure 7C, Hat1 was transiently associated with nascent chromatin. Hat1 was retained on chromatin longer than PCNA suggesting that Hat1 was likely not associated with the DNA replication machinery. Hat1 appeared to dissociate from nascent chromatin during the time period where chromatin maturation occurs. Indeed, the loss of Hat1 preceded the loss of H4 lysine 5 and lysine 12 acetylation. The observation that dissociation of Hat1 from chromatin precedes deacetylation of H4 lysines 5 and 12 suggests that Hat1 may be directly regulating chromatin maturation by acting to maintain the acetylation state of newly synthesized histone H4.

DISCUSSION

While the di-acetylation of newly synthesized histone H4 on lysine 5 and 12 was discovered >40 years ago, the function of this mark in the process of chromatin assembly has remained an open question (48,50,111–113). Using $Hat1^{+/+}$ and $Hat1^{-/-}$ MEF cell lines we recently demonstrated that Hat1 is required for the acetylation of lysines 5 and 12 on histone H4 molecules deposited during replication-coupled chromatin assembly. We have now used this model system to directly analyze the effect of Hat1 on the assembly of nascent chromatin.

A number of reports have suggested that the acetylation of newly synthesized histone H4 plays a direct role in facilitating the nuclear import of H3/H4 dimers. Experiments in both *S. cerevisiae* and mammalian cells using fusions of the H4 NH_2-terminal tail to fluorescent proteins show that the H4 tail can direct nuclear import of the fusion protein. Results in yeast show that mutating lysines 5, 8, 12 and 16 to alanine significantly decreased import into the nucleus (78). Conversely, in HeLa cells, mutating H4 lysines 5 and 12 to glutamine, which mimics the acetylation of these residues, enhances nuclear import of the fusion. Consistent with this observation, acetylation of lysines 5 and 12 enhances the binding of synthetic H4 NH_2-terminal tail peptides to the histone nuclear import factor Importin-4 (114). Mutation of lysines 5 and 12 were also shown to influence the import of recombinant H4 proteins into the nuclei of *P. polysephalum*. While recombinant wild type or lysine 5 and 12 to glutamine mutants were efficiently imported into the nucleus, lysine 5 and 12 to arginine mutants were not (70). However, a recent comprehensive analysis of the binding of H4 tail peptides to a wide range of importins showed that acetylation of H4 lysine 5 and 12 had little impact on the binding of the H4 tail to any of the importins tested (115). Our results are consistent with the latter analysis and suggest that the Hat1-dependent acetylation of newly synthesized histone H4 does not have a significant effect on nuclear import of this histone. While the assay we used may not have been capable of detecting subtle changes in nuclear import in the $Hat1^{-/-}$ cells, the lack of a defect in histone deposition in these cells suggests that any potential decrease in nuclear import is not functionally relevant. There may be a number of reasons to explain the discrepancies in these results. For example, H4 NH_2-terminal tail fusion proteins and recombinant histones may not be imported into the nucleus using the same pathway used by endogenous H3/H4 complexes.

The role of Hat1 in the replication-coupled deposition of histones H3 and H4 is typically described as a transient one. The newly synthesized H3 and H4 interact with chaperones that facilitate the formation of dimers. These dimers then interact with the Hat1/Rbap46 complex, which acetylates H4 on lysines 5 and 12. This modified H3/H4 complex is then passed to Asf1, which interacts with specific Importins to bring the histones into the nucleus. Following additional acetylation, the H3/H4 complexes are then passed to other histone chaperones, such as CAF-1, which then results in their deposition onto newly synthesized DNA.

Our results suggest that Hat1 plays a much more extensive role in this process. As depicted in Figure 7D, we propose that the Hat1/Rbap46 complex does not dissociate from histone H4 following its acetylation. Rather, Hat1 remains associated with H3/H4 throughout the process of nuclear import and histone deposition. It is likely that the Hat1/Rbap46/H3/H4 complex functions in the Asf1-dependent pathway as it has been demonstrated that the Hat1/Rbap46 complex and Asf1 can bind H3/H4 simultaneously (8,15). It is possible that the Hat1/Rbap46/H3/H4 complex may also participate in Asf1-independent histone import and deposition pathways, perhaps through association with the histone chaperone NASP (61,67,116). Following deposition onto newly replicated DNA, the Hat1/Rbap46 complex remains bound to the newly deposited histones. The mechanism by which the Hat1/Rbap46 dissociates from nascent chromatin is not clear but it appears that chromatin maturation, at least in terms of the deacetylation of new H4 lysines 5 and 12, does not proceed until this dissociation occurs. Hence, the Hat1-

dependent acetylation of newly synthesized histone H4 may function to regulate both nascent chromatin assembly and maturation.

The prolonged association of the Hat1/Rbap46 complex with newly synthesized H3 and H4 is consistent with numerous proteomic studies of soluble H3/H4 complexes. Soluble forms of histone H3 or H4 have been purified from cytosolic and nuclear extracts and from a variety of different eukaryotic organisms (8,18,67–73,114). In each case, Hat1 and Rbap46 (Hat2) were found to be prominent components of these soluble histone complexes. Clearly, the Hat1/Rbap46 complex is involved in stable associations with histones H3 and H4 when they are not incorporated into chromatin. The stable association of the Hat1/Rbap46 complex with soluble H3/H4 complexes may also provide a rationale for the recent observation that loss of Hat1 impacts the acetylation state of histone H3 during replication-coupled chromatin assembly (13). While Hat1 does not have enzymatic activity toward histone H3, the association of H3 with the Hat1/Rbap46 complex may be required for the subsequent acetylation of histone H3.

Proteomic comparisons of nascent chromatin assembled in the presence and absence of Hat1 indicate that Hat1 is not required for the physical association of the core histones with chromatin but, rather, that the structure of the chromatin formed in the absence of Hat1 is defective. This is indicated by alterations in the abundance on nascent chromatin of multiple factors that influence DNA topology and chromatin structure. Our results suggest a number of mechanisms by which Hat1 may be influencing the structure and topology of nascent chromatin. First, the Hat1-dependent acetylation of histone H4 may directly target bromodomain-containing chromatin modifying activities to the newly deposited histones. Indeed, Brd3 and Brg1 display specificity for binding to H4 NH_2-terminal tail peptides that contain lysine 5 and/or lysine 12 acetylation (Figure 7E). A recent iPOND quantitative proteomics analysis found that, in addition to Brd3, Brd2 and Brd4 are also components of nascent chromatin (83). While we did not detect Brd2 and Brd4, it is likely that our effort to minimize false positives by only including proteins identified in all biological replicates also resulted in the loss of some true positives from our dataset. Efforts to directly assess the effect of Hat1 loss on the association of Brd2 and Brd4 with nascent chromatin have been hindered by technical difficulties arising from the effect of the iPOND procedure on the antigenicity of some proteins. While the function of Brd3 is not known, the depletion of Brg1 and Baz1a could clearly alter the structure and topology of the newly assembled chromatin. For example, a decrease in the Baz1a-containing ACF complex on nascent chromatin could result in nucleosomes that are more tightly packed. Alternatively, the decrease in ACF could result in the accumulation of prenucleosomes that are not converted into canonical nucleosomes in a timely fashion (117). Either of these possibilities is consistent with the shorter repeat length and the enrichment of core histones observed in nascent chromatin from the $Hat1^{-/-}$ cells. The depletion of Brg1 on nascent chromatin in the absence of Hat1 may also trigger a compensatory enhancement of Topoisomerase 2 recruitment as these proteins function synergistically in the formation of facultative heterochromatin (118). Second, the physical association of the Hat1/Rbap46 complex with the newly synthesized histones during their deposition onto DNA may play a direct role in orchestrating the wrapping of the DNA onto the H3/H4 tetramer. This suggests that it may not be just the enzymatic activity, but also the histone chaperone function of the Hat1/Rbap46 complex, that is involved in regulating the assembly and maturation of nascent chromatin (119). Relatedly, the presence of the Hat1/Rbap46 complex on newly assembled nucleosomes may influence the association of neighboring nucleosomes, either through steric hindrance of through blocking the electrostatic interactions between the H4 NH_2-terminal tails and the acidic patch on the face of adjacent nucleosomes (120). Finally, Hat1 may be involved in the acetylation of non-histone components of nascent chromatin. The localization of these factors and their subsequent influence on nascent chromatin structure could be altered by the presence or absence of Hat1-dependent acetylation.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Das,C., Tyler,J.K. and Churchill,M.E. (2010) The histone shuffle: histone chaperones in an energetic dance. *Trends Biochem. Sci.*, **35**, 476–489.
2. Alabert,C. and Groth,A. (2012) Chromatin replication and epigenome maintenance. *Nat. Rev. Mol. Cell. Biol.*, **13**, 153–167.
3. Annunziato,A.T. (2012) Assembling chromatin: the long and winding road. *Biochim. Biophys. Acta*, **1819**, 196–210.
4. Gurard-Levin,Z.A., Quivy,J.P. and Almouzni,G. (2014) Histone chaperones: assisting histone traffic and nucleosome dynamics. *Annu. Rev. Biochem.*, **83**, 487–517.
5. Loyola,A., Bonaldi,T., Roche,D., Imhof,A. and Almouzni,G. (2006) PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state. *Mol. Cell*, **24**, 309–316.
6. Rivera,C., Gurard-Levin,Z.A., Almouzni,G. and Loyola,A. (2014) Histone lysine methylation and chromatin replication. *Biochim. Biophys. Acta*, **1839**, 1433–1439.
7. Rivera,C., Saavedra,F., Alvarez,F., Díaz-Celis,C., Ugalde,V., Li,J., Forné,I., Gurard-Levin,Z.A., Almouzni,G., Imhof,A. *et al.* (2015) Methylation of histone H3 lysine 9 occurs during translation. *Nucleic Acids Res.*, **43**, 9097–9106.
8. Campos,E.I., Fillingham,J., Li,G., Zheng,H., Voigt,P., Kuo,W.H., Seepany,H., Gao,Z., Day,L.A., Greenblatt,J.F. *et al.* (2010) The program for processing newly synthesized histones H3.1 and H4. *Nat. Struct. Mol. Biol.*, **17**, 1343–1351.
9. Alvarez,F., Munoz,F., Schilcher,P., Imhof,A., Almozuni,G. and Loyola,A. (2011) Sequential establishment of marks on soluble histones H3 and H4. *J. Biol. Chem.*, **286**, 17714–17721.
10. Parthun,M.R., Widom,J. and Gottschling,D.E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell*, **87**, 85–94.

11. Kleff,S., Andrulis,E.D., Anderson,C.W. and Sternglanz,R. (1995) Identification of a gene encoding a yeast histone H4 acetyltransferase. *J. Biol. Chem.*, **270**, 24674–24677.
12. Verreault,A., Kaufman,P.D., Kobayashi,R. and Stillman,B. (1998) Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. *Curr. Biol.*, **8**, 96–108.
13. Nagarajan,P., Ge,Z., Sirbu,B., Doughty,C., Agudelo Garcia,P.A., Schlederer,M., Annunziato,A.T., Cortez,D., Kenner,L. and Parthun,M.R. (2013) Histone acetyl transferase 1 is essential for mammalian development, genome stability, and the processing of newly synthesized histones H3 and H4. *PLoS Genet.*, **9**, e1003518.
14. Chang,L., Loranger,S.S., Mizzen,C., Ernst,S.G., Allis,C.D. and Annunziato,A.T. (1997) Histones in transit: cytosolic histone complexes and diacetylation of H4 during nucleosome assembly in human cells. *Biochemistry*, **36**, 469–480.
15. Fillingham,J., Recht,J., Silva,A.C., Suter,B., Emili,A., Stagljar,I., Krogan,N.J., Allis,C.D., Keogh,M.C. and Greenblatt,J.F. (2008) Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. *Mol. Cell. Biol.*, **28**, 4342–4353.
16. Blackwell,J.S. Jr, Wilkinson,S.T., Mosammaparast,N. and Pemberton,L.F. (2007) Mutational analysis of H3 and H4 N termini reveals distinct roles in nuclear import. *J. Biol. Chem.*, **282**, 20142–20150.
17. Mosammaparast,N., Ewart,C.S. and Pemberton,L.F. (2002) A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. *EMBO J.*, **21**, 6527–6538.
18. Barman,H.K., Takami,Y., Nishijima,H., Shibahara,K., Sanematsu,F. and Nakayama,T. (2008) Histone acetyltransferase-1 regulates integrity of cytosolic histone H3-H4 containing complex. *Biochem. Biophys. Res. Commun.*, **373**, 624–630.
19. Adkins,M.W., Carson,J.J., English,C.M., Ramey,C.J. and Tyler,J.K. (2007) The histone chaperone anti-silencing function 1 stimulates the acetylation of newly synthesized histone H3 in S-phase. *J. Biol. Chem.*, **282**, 1334–1340.
20. Burgess,R.J., Zhou,H., Han,J. and Zhang,Z. (2010) A role for Gcn5 in replication-coupled nucleosome assembly. *Mol. Cell*, **37**, 469–480.
21. Chen,C.C., Carson,J.J., Feser,J., Tamburini,B., Zabaronick,S., Linger,J. and Tyler,J.K. (2008) Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell*, **134**, 231–243.
22. Ge,Z., Nair,D., Guan,X., Rastogi,N., Freitas,M.A. and Parthun,M.R. (2013) Sites of acetylation on newly synthesized histone h4 are required for chromatin assembly and DNA damage response signaling. *Mol. Cell. Biol.*, **33**, 3286–3298.
23. Han,J., Zhou,H., Horazdovsky,B., Zhang,K., Xu,R.M. and Zhang,Z. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science*, **315**, 653–655.
24. Han,J., Zhou,H., Li,Z., Xu,R.M. and Zhang,Z. (2007) Acetylation of lysine 56 of histone H3 catalyzed by RTT109 and regulated by ASF1 is required for replisome integrity. *J. Biol. Chem.*, **282**, 28587–28596.
25. Han,J., Zhou,H., Li,Z., Xu,R.M. and Zhang,Z. (2007) The Rtt109-Vps75 histone acetyltransferase complex acetylates non-nucleosomal histone H3. *J. Biol. Chem.*, **282**, 14158–14164.
26. Kaplan,T., Liu,C.L., Erkmann,J.A., Holik,J., Grunstein,M., Kaufman,P.D., Friedman,N. and Rando,O.J. (2008) Cell cycle- and chaperone-mediated regulation of H3K56ac incorporation in yeast. *PLoS Genet.*, **4**, e1000270.
27. Recht,J., Tsubota,T., Tanny,J.C., Diaz,R.L., Berger,J.M., Zhang,X., Garcia,B.A., Shabanowitz,J., Burlingame,A.L., Hunt,D.F. *et al.* (2006) Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 6988–6993.
28. Tsubota,T., Berndsen,C.E., Erkmann,J.A., Smith,C.L., Yang,L., Freitas,M.A., Denu,J.M. and Kaufman,P.D. (2007) Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. *Mol. Cell*, **25**, 703–712.
29. Das,C., Lucia,M.S., Hansen,K.C. and Tyler,J.K. (2009) CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature*, **459**, 113–117.
30. Li,Q., Zhou,H., Wurtele,H., Davies,B., Horazdovsky,B., Verreault,A. and Zhang,Z. (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell*, **134**, 244–255.
31. Hoek,M. and Stillman,B. (2003) Chromatin assembly factor 1 is essential and couples chromatin assembly to DNA replication in vivo. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 12183–12188.
32. Malay,A.D., Umehara,T., Matsubara-Malay,K., Padmanabhan,B. and Yokoyama,S. (2008) Crystal structures of fission yeast histone chaperone Asf1 complexed with the Hip1 B-domain or the Cac2 C terminus. *J. Biol. Chem.*, **283**, 14022–14031.
33. Mello,J.A., Sillje,H.H., Roche,D.M., Kirschner,D.B., Nigg,E.A. and Almouzni,G. (2002) Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. *EMBO Rep.*, **3**, 329–334.
34. Moggs,J.G., Grandi,P., Quivy,J.P., Jonsson,Z.O., Hubscher,U., Becker,P.B. and Almouzni,G. (2000) A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.*, **20**, 1206–1218.
35. Ridgway,P. and Almouzni,G. (2000) CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair. *J. Cell Sci.*, **113**, 2647–2658.
36. Shibahara,K. and Stillman,B. (1999) Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell*, **96**, 575–585.
37. Tyler,J.K., Collins,K.A., Prasad-Sinha,J., Amiott,E., Bulger,M., Harte,P.J., Kobayashi,R. and Kadonaga,J.T. (2001) Interaction between the Drosophila CAF-1 and ASF1 chromatin assembly factors. *Mol. Cell. Biol.*, **21**, 6574–6584.
38. Liu,W.H., Roemer,S.C., Port,A.M. and Churchill,M.E. (2012) CAF-1-induced oligomerization of histones H3/H4 and mutually exclusive interactions with Asf1 guide H3/H4 transitions among histone chaperones and DNA. *Nucleic Acids Res.*, **40**, 11229–11239.
39. Winkler,D.D., Zhou,H., Dar,M.A., Zhang,Z. and Luger,K. (2012) Yeast CAF-1 assembles histone (H3-H4)2 tetramers prior to DNA deposition. *Nucleic Acids Res.*, **40**, 10139–10149.
40. Mosammaparast,N., Jackson,K.R., Guo,Y., Brame,C.J., Shabanowitz,J., Hunt,D.F. and Pemberton,L.F. (2001) Nuclear import of histone H2A and H2B is mediated by a network of karyopherins. *J. Cell Biol.*, **153**, 251–262.
41. Annunziato,A.T., Schindler,R.K., Thomas,C.A. Jr and Seale,R.L. (1981) Dual nature of newly replicated chromatin. Evidence for nucleosomal and non-nucleosomal DNA at the site of native replication forks. *J. Biol. Chem.*, **256**, 11880–11886.
42. Annunziato,A.T., Schindler,R.K., Riggs,M.G. and Seale,R.L. (1982) Association of newly synthesized histones with replicating and nonreplicating regions of chromatin. *J. Biol. Chem.*, **257**, 8507–8515.
43. Annunziato,A.T. and Seale,R.L. (1982) Maturation of nucleosomal and nonnucleosomal components of nascent chromatin: differential requirements for concurrent protein synthesis. *Biochemistry*, **21**, 5431–5438.
44. Annunziato,A.T. and Seale,R.L. (1983) Histone deacetylation is required for the maturation of newly replicated chromatin. *J. Biol. Chem.*, **258**, 12675–12684.
45. Perry,C.A. and Annunziato,A.T. (1991) Histone acetylation reduces H1-mediated nucleosome interactions during chromatin assembly. *Exp. Cell Res.*, **196**, 337–345.
46. Benson,L.J., Gu,Y., Yakovleva,T., Tong,K., Barrows,C., Strack,C.L., Cook,R.G., Mizzen,C.A. and Annunziato,A.T. (2006) Modifications of H3 and H4 during chromatin replication, nucleosome assembly, and histone exchange. *J. Biol. Chem.*, **281**, 9287–9296.
47. Allis,C.D., Chicoine,L.G., Richman,R. and Schulman,I.G. (1985) Deposition-related histone acetylation in micronuclei of conjugating Tetrahymena. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 8048–8052.
48. Chicoine,L.G., Schulman,I.G., Richman,R., Cook,R.G. and Allis,C.D. (1986) Nonrandom utilization of acetylation sites in histones isolated from Tetrahymena. Evidence for functionally distinct H4 acetylation sites. *J. Biol. Chem.*, **261**, 1071–1076.
49. Sobel,R.E., Cook,R.G. and Allis,C.D. (1994) Non-random acetylation of histone H4 by a cytoplasmic histone acetyltransferase as determined by novel methodology. *J. Biol. Chem.*, **269**, 18576–18582.
50. Sobel,R.E., Cook,R.G., Perry,C.A., Annunziato,A.T. and Allis,C.D. (1995) Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 1237–1241.

51. Polo,S.E., Roche,D. and Almouzni,G. (2006) New histone incorporation marks sites of UV repair in human cells. *Cell*, **127**, 481–493.
52. Annunziato,A.T. and Hansen,J.C. (2000) Role of histone acetylation in the assembly and modulation of chromatin structures. *Gene Expr.*, **9**, 37–61.
53. Chapman,J.R. and Jackson,S.P. (2008) Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep.*, **9**, 795–801.
54. Megee,P.C., Morgan,B.A., Mittman,B.A. and Smith,M.M. (1990) Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. *Science*, **247**, 841–845.
55. Zhang,W., Bone,J.R., Edmondson,D.G., Turner,B.M. and Roth,S.Y. (1998) Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. *EMBO J.*, **17**, 3155–3167.
56. Ma,X.J., Wu,J., Altheim,B.A., Schultz,M.C. and Grunstein,M. (1998) Deposition-related sites K5/K12 in histone H4 are not required for nucleosome deposition in yeast. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 6693–6698.
57. Shibahara,K., Verreault,A. and Stillman,B. (2000) The N-terminal domains of histones H3 and H4 are not necessary for chromatin assembly factor-1- mediated nucleosome assembly onto replicated DNA in vitro. *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 7766–7771.
58. Ruiz-García,A.B., Sendra,R., Galiana,M., Pamblanco,M., Pérez-Ortín,J.E. and Tordera,V. (1998) HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. *J. Biol. Chem.*, **273**, 12599–12605.
59. Qin,S. and Parthun,M.R. (2002) Histone H3 and the histone acetyltransferase Hat1p contribute to DNA double-strand break repair. *Mol. Cell. Biol.*, **22**, 8353–8365.
60. Kelly,T.J., Qin,S., Gottschling,D.E. and Parthun,M.R. (2000) Type B histone acetyltransferase Hat1p participates in telomeric silencing. *Mol. Cell. Biol.*, **20**, 7051–7058.
61. Ge,Z., Wang,H. and Parthun,M.R. (2011) Nuclear Hat1p complex (NuB4) components participate in DNA repair-linked chromatin reassembly. *J. Biol. Chem.*, **286**, 16790–16799.
62. Benson,L.J., Phillips,J.A., Gu,Y., Parthun,M.R., Hoffman,C.S. and Annunziato,A.T. (2007) Properties of the type B histone acetyltransferase Hat1: H4 tail interaction, site preference, and involvement in DNA repair. *J. Biol. Chem.*, **282**, 836–842.
63. Tong,K., Keller,T., Hoffman,C.S. and Annunziato,A.T. (2012) Schizosaccharomyces pombe Hat1 (Kat1) is associated with Mis16, and is required for telomeric silencing. *Eukaryot. Cell*, **11**, 1095–1103.
64. Tscherner,M., Stappler,E., Hnisz,D. and Kuchler,K. (2012) The histone acetyltransferase Hat1 facilitates DNA damage repair and morphogenesis in Candida albicans. *Mol. Microbiol.*, **86**, 1197–1214.
65. Barman,H.K., Takami,Y., Ono,T., Nishijima,H., Sanematsu,F., Shibahara,K. and Nakayama,T. (2006) Histone acetyltransferase 1 is dispensable for replication-coupled chromatin assembly but contributes to recover DNA damages created following replication blockage in vertebrate cells. *Biochem. Biophys. Res. Commun.*, **345**, 1547–1557.
66. Yang,X., Li,L., Liang,J., Shi,L., Yang,J., Yi,X., Zhang,D., Han,X., Yu,N. and Shang,Y. (2013) Histone acetyltransferase 1 promotes homologous recombination in DNA repair by facilitating histone turnover. *J. Biol. Chem.*, **288**, 18271–18282.
67. Ai,X. and Parthun,M.R. (2004) The nuclear Hat1p/Hat2p complex: a molecular link between type B histone acetyltransferases and chromatin assembly. *Mol. Cell*, **14**, 195–205.
68. Tagami,H., Ray-Gallet,D., Almouzni,G. and Nakatani,Y. (2004) Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell*, **116**, 51–61.
69. Saade,E., Mechold,U., Kulyyassov,A., Vertut,D., Lipinski,M. and Ogryzko,V. (2009) Analysis of interaction partners of H4 histone by a new proteomics approach. *Proteomics*, **9**, 4934–4943.
70. Ejlassi-Lassallette,A., Mocquard,E., Arnaud,M.C. and Thiriet,C. (2011) H4 replication-dependent diacetylation and Hat1 promote S-phase chromatin assembly in vivo. *Mol. Biol. Cell*, **22**, 245–255.
71. Mosammaparast,N., Guo,Y., Shabanowitz,J., Hunt,D.F. and Pemberton,L.F. (2002) Pathways mediating the nuclear import of histones H3 and H4 in yeast. *J. Biol. Chem.*, **277**, 862–868.
72. Drane,P., Ouararhni,K., Depaux,A., Shuaib,M. and Hamiche,A. (2010) The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. *Genes Dev.*, **24**, 1253–1265.
73. Campos,E.I., Smits,A.H., Kang,Y.H., Landry,S., Escobar,T.M., Nayak,S., Ueberheide,B.M., Durocher,D., Vermeulen,M., Hurwitz,J. *et al.* (2015) Analysis of the histone H3.1 interactome: a suitable chaperone for the right event. *Mol. Cell*, **60**, 697–709.
74. Cox,J. and Mann,M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.*, **26**, 1367–1372.
75. Annunziato,A.T. (2013) Assembling chromatin: the long and winding road. *Biochim. Biophys. Acta*, **1819**, 196–210.
76. Parthun,M.R. (2007) Hat1: the emerging cellular roles of a type B histone acetyltransferase. *Oncogene*, **26**, 5319–5328.
77. Parthun,M.R. (2012) Histone acetyltransferase 1: More than just an enzyme? *Biochim. Biophys. Acta*, **1819**, 256–263.
78. Glowczewski,L., Waterborg,J.H. and Berman,J.G. (2004) Yeast chromatin assembly complex 1 protein excludes nonacetylatable forms of histone H4 from chromatin and the nucleus. *Mol. Cell. Biol.*, **24**, 10180–10192.
79. Fillingham,J. and Greenblatt,J.F. (2008) A histone code for chromatin assembly. *Cell*, **134**, 206–208.
80. Sirbu,B.M., Couch,F.B., Feigerle,J.T., Bhaskara,S., Hiebert,S.W. and Cortez,D. (2011) Analysis of protein dynamics at active, stalled, and collapsed replication forks. *Genes Dev.*, **25**, 1320–1327.
81. Kliszczak,A.E., Rainey,M.D., Harhen,B., Boisvert,F.M. and Santocanale,C. (2011) DNA mediated chromatin pull-down for the study of chromatin replication. *Sci. Rep.*, **1**, 95.
82. Alabert,C., Bukowski-Wills,J.C., Lee,S.B., Kustatscher,G., Nakamura,K., de Lima Alves,F., Menard,P., Mejlvang,J., Rappsilber,J. and Groth,A. (2014) Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat. Cell Biol.*, **16**, 281–293.
83. Dungrawala,H., Rose,K.L., Bhat,K.P., Mohni,K.N., Glick,G.G., Couch,F.B. and Cortez,D. (2015) The replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Mol. Cell*, **59**, 998–1010.
84. Sirbu,B.M., McDonald,W.H., Dungrawala,H., Badu-Nkansah,A., Kavanaugh,G.M., Chen,Y., Tabb,D.L. and Cortez,D. (2013) Identification of proteins at active, stalled, and collapsed replication forks using isolation of proteins on nascent DNA (iPOND) coupled with mass spectrometry. *J. Biol. Chem.*, **288**, 31458–31467.
85. Wang,W.J., Tay,H.G., Soni,R., Perumal,G.S., Goll,M.G., Macaluso,F.P., Asara,J.M., Amack,J.D. and Tsou,M.F. (2013) CEP162 is an axoneme-recognition protein promoting ciliary transition zone assembly at the cilia base. *Nat. Cell Biol.*, **15**, 591–601.
86. Morito,D., Nishikawa,K., Hoseki,J., Kitamura,A., Kotani,Y., Kiso,K., Kinjo,M., Fujiyoshi,Y. and Nagata,K. (2014) Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. *Sci. Rep.*, **4**, 4442.
87. Kamada,F., Aoki,Y., Narisawa,A., Abe,Y., Komatsuzaki,S., Kikuchi,A., Kanno,J., Niihori,T., Ono,M., Ishii,N. *et al.* (2011) A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. *J. Hum. Genet.*, **56**, 34–40.
88. Liu,W., Morito,D., Takashima,S., Mineharu,Y., Kobayashi,H., Hitomi,T., Hashikata,H., Matsuura,N., Yamazaki,S., Toyoda,A. *et al.* (2011) Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. *PLoS One*, **6**, e22542.
89. Khavari,P.A., Peterson,C.L., Tamkun,J.W., Mendel,D.B. and Crabtree,G.R. (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature*, **366**, 170–174.
90. Li,G., White,C.A., Lam,T., Pone,E.J., Tran,D.C., Hayama,K.L., Zan,H., Xu,Z. and Casali,P. (2013) Combinatorial H3K9acS10ph histone modification in IgH locus S regions targets 14-3-3 adaptors

and AID to specify antibody class-switch DNA recombination. *Cell Rep.*, **5**, 702–714.
91. Mortenson,J.B., Heppler,L.N., Banks,C.J., Weerasekara,V.K., Whited,M.D., Piccolo,S.R., Johnson,W.E., Thompson,J.W. and Andersen,J.L. (2015) Histone deacetylase 6 (HDAC6) promotes the pro-survival activity of 14-3-3zeta via deacetylation of lysines within the 14-3-3zeta binding pocket. *J. Biol. Chem.*, **290**, 12487–12496.
92. Chen,F. and Wagner,P.D. (1994) 14-3-3 proteins bind to histone and affect both histone phosphorylation and dephosphorylation. *FEBS Lett.*, **347**, 128–132.
93. Winter,S., Simboeck,E., Fischle,W., Zupkovitz,G., Dohnal,I., Mechtler,K., Ammerer,G. and Seiser,C. (2008) 14-3-3 proteins recognize a histone code at histone H3 and are required for transcriptional activation. *EMBO J.*, **27**, 88–99.
94. Wang,C., Yao,C., Li,Y., Cai,W., Bao,X., Girton,J., Johansen,J. and Johansen,K.M. (2013) Evidence against a role for the JIL-1 kinase in H3S28 phosphorylation and 14-3-3 recruitment to active genes in Drosophila. *PLoS One*, **8**, e62484.
95. Wade,P.A., Jones,P.L., Vermaak,D. and Wolffe,A.P. (1998) A multiple subunit Mi-2 histone deacetylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. *Curr. Biol.*, **8**, 843–846.
96. Wade,P.A., Gegonne,A., Jones,P.L., Ballestar,E., Aubry,F. and Wolffe,A.P. (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nat. Genet.*, **23**, 62–66.
97. Jacobson,R.H., Ladurner,A.G., King,D.S. and Tjian,R. (2000) Structure and function of a human TAFII250 double bromodomain module. *Science*, **288**, 1422–1425.
98. Owen,D.J., Ornaghi,P., Yang,J.C., Lowe,N., Evans,P.R., Ballario,P., Neuhaus,D., Filetici,P. and Travers,A.A. (2000) The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO J.*, **19**, 6141–6149.
99. Hudson,B.P., Martinez-Yamout,M.A., Dyson,H.J. and Wright,P.E. (2000) Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. *J. Mol. Biol.*, **304**, 355–370.
100. LeRoy,G., Loyola,A., Lane,W.S. and Reinberg,D. (2000) Purification and characterization of a human factor that assembles and remodels chromatin. *J. Biol. Chem.*, **275**, 14787–14790.
101. Poot,R.A., Dellaire,G., Hulsmann,B.B., Grimaldi,M.A., Corona,D.F., Becker,P.B., Bickmore,W.A. and Varga-Weisz,P.D. (2000) HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. *EMBO J.*, **19**, 3377–3387.
102. LeRoy,G., Rickards,B. and Flint,S.J. (2008) The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. *Mol. Cell*, **30**, 51–60.
103. Nitiss,J.L. (2009) DNA topoisomerase II and its growing repertoire of biological functions. *Nat. Rev. Cancer*, **9**, 327–337.
104. Brackley,C.A., Allan,J., Keszenman-Pereyra,D. and Marenduzzo,D. (2015) Topological constraints strongly affect chromatin reconstitution in silico. *Nucleic Acids Res.*, **43**, 63–73.
105. Tanabe,K., Ikegami,Y., Ishida,R. and Andoh,T. (1991) Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res.*, **51**, 4903–4908.
106. Daniel,J.M. and Reynolds,A.B. (1999) The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. *Mol. Cell. Biol.*, **19**, 3614–3623.
107. Prokhortchouk,A., Hendrich,B., Jorgensen,H., Ruzov,A., Wilm,M., Georgiev,G., Bird,A. and Prokhortchouk,E. (2001) The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev.*, **15**, 1613–1618.
108. Daniel,J.M., Spring,C.M., Crawford,H.C., Reynolds,A.B. and Baig,A. (2002) The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. *Nucleic Acids Res.*, **30**, 2911–2919.
109. Kim,S.W., Fang,X., Ji,H., Paulson,A.F., Daniel,J.M., Ciesiolka,M., van Roy,F. and McCrea,P.D. (2002) Isolation and characterization of XKaiso, a transcriptional repressor that associates with the catenin Xp120(ctn) in Xenopus laevis. *J. Biol. Chem.*, **277**, 8202–8208.
110. Jones,P.L., Veenstra,G.J., Wade,P.A., Vermaak,D., Kass,S.U., Landsberger,N., Strouboulis,J. and Wolffe,A.P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat. Genet.*, **19**, 187–191.
111. Ruiz-Carillo,A., Wangh,L.J. and Allfry,V. (1975) Processing of newly synthesized histone molecules. *Science*, **190**, 117–128.
112. Louie,A.J., Candido,E.P. and Dixon,G.H. (1974) Enzymatic modifications and their possible roles in regulating the binding of basic proteins to DNA and in controlling chromosomal structure. *Cold Spring Harb. Symp. Quant. Biol.*, **38**, 803–819.
113. Jackson,V., Shires,A., Tanphaichitr,N. and Chalkley,R. (1976) Modifications to histones immediately after synthesis. *J. Mol. Biol.*, **104**, 471–483.
114. Alvarez,F., Munoz,F., Schilcher,P., Imhof,A., Almouzni,G. and Loyola,A. (2011) Sequential establishment of marks on soluble histones H3 and H4. *J. Biol. Chem.*, **286**, 17714–17721.
115. Soniat,M., Cagatay,T. and Chook,Y.M. (2016) Recognition elements in the histone H3 and H4 tails for seven different Importins. *J. Biol. Chem.*, **291**, 21171–21183.
116. Poveda,A., Pamblanco,M., Tafrov,S., Tordera,V., Sternglanz,R. and Sendra,R. (2004) Hif1 is a component of yeast histone acetyltransferase B, a complex mainly localized in the nucleus. *J. Biol. Chem.*, **279**, 16033–16043.
117. Fei,J., Torigoe,S.E., Brown,C.R., Khuong,M.T., Kassavetis,G.A., Boeger,H. and Kadonaga,J.T. (2015) The prenucleosome, a stable conformational isomer of the nucleosome. *Genes Dev.*, **29**, 2563–2575.
118. Miller,E.L., Hargreaves,D.C., Kadoch,C., Chang,C.Y., Calarco,J.P., Hodges,C., Buenrostro,J.D., Cui,K., Greenleaf,W.J., Zhao,K. *et al.* (2017) TOP2 synergizes with BAF chromatin remodeling for both resolution and formation of facultative heterochromatin. *Nat. Struct. Mol. Biol.*, **24**, 344–352.
119. Parthun,M.R. (2013) Histone acetyltransferase 1: more than just an enzyme? *Biochim. Biophys. Acta*, **1819**, 256–263.
120. Luger,K., Mader,A.W., Richmond,R.K., Sargent,D.F. and Richmond,T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389**, 251–260.