DNA and histone modifications direct the functional state of chromatin and thereby the readout of the genome. Candidate approaches and histone peptide affinity purification experiments have identified several proteins that bind to chromatin marks. However, the complement of factors that is recruited by individual and combinations of DNA and histone modifications has not yet been defined. Here, we present a strategy based on recombinant, uniformly modified chromatin templates used in affinity purification experiments in conjunction with SILAC-based quantitative mass spectrometry for this purpose. On the prototypic H3K4me3 and H3K9me3 histone modification marks we compare our method with a histone N-terminal peptide affinity purification approach. Our analysis shows that only some factors associate with both, chromatin and peptide matrices but that a surprisingly large number of proteins differ in their association with these templates. Global analysis of the proteins identified implies specific domains mediating recruitment to the chromatin marks. Our proof-of-principle studies show that chromatin templates with defined modification patterns can be used to decipher how the histone code is read and translated. Molecular & Cellular Proteomics 10: 10.1074/mcp.M110.005371, 1–16, 2011.

DNA methylation and histone post-translational modifications (PTM) play important roles in regulating chromatin states and thereby the use and readout of the genome. Tri-methylation of lysine 4 (H3K4me3) and lysine 9 (H3K9me3) of histone H3 have, for example, been connected to transcriptional activation and repression, respectively. They therefore present a prototypic pair of antagonistic histone PTMs.

Generally, chromatin marks either influence chromatin packaging directly or via recruitment of specific proteins and multi-protein complexes that mediate downstream effects (1, 2). Candidate approaches of individual factors or using targeted libraries of protein families together with histone tail peptide affinity purification experiments carried out in isolation or on peptide arrays have identified a number of proteins that specifically interact with individual chromatin marks (see for example refs. 3–6). These include factors containing methyl-DNA binding domains as well as chromodomains, plant homeodomain (PHD) fingers, tudor domains, and ankyrin repeats interacting with histone methyl-lysine residues. Further, 14-3-3 proteins interacting with histone phospho-serine residues and bromodomodaining factors binding to histone acetyl-lysine residues have been described (7). In vitro studies have characterized the exact binding specificities of several proteins containing these domains. Also, structural insights are now available for a number of chromatin mark binding complexes (7, 8).

Interestingly, the interactions of individual domains of chromatin modification binding proteins with their cognate marks

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are rather weak (interaction strength in the micromolar range) (9). Although the study of interactions of individual proteins with DNA methylation or distinct histone PTM marks has been central to our current understanding of chromatin mediated processes, it is emerging that patterns of marks rather than individual modifications direct functional states of chromatin (10, 11). Here, factors containing multiple domains interacting with different chromatin marks have gained high interest (12). Multivalent binding might not only allow for stronger and thereby more discriminatory interaction than single domain binding, but could also direct readout of complex patterns of modifications. Also, multiprotein complexes appear to contain several factors with the same or distinct chromatin mark recognition functionality thereby possibly establishing more stable interaction.

Gaining global insight into the relationship of chromatin modifications and functional states of chromatin ultimately requires isolation and characterization of intact chromatin domains from cells. In absence of such experimental systems in vitro approaches that mimic and incorporate different DNA methylation and histone PTM configurations will likely be extremely useful in defining the complement of factors that targets a given pattern of chromatin marks. Here, DNA and/or histone tail peptide affinity purification experiments can only be of limited value as only individual or shortly spaced combinatorial patterns of modifications can be analyzed (see for example ref. 13).

Nonquantitative mass spectrometry (e.g. MudPIT, ref. 14) analysis of differential affinity purification reactions has been useful in identifying proteins binding a given target (4). However, because these methods do not provide sufficient quantitative information on the proteins recovered in separate experiments in the first place, factors that bind two separate matrices (e.g. sample and control) with different strength will not be necessarily recognized as specific interaction partners of either one. Therefore, different mass spectrometry methods have been introduced that allow identification and sensitive quantification of proteins in matched experiments (15). Especially, isotope labeling by amino acids in cell culture (SILAC) has proven useful in various proteomics based approaches (16). Here, we set out to establish an in vitro system usable for the analysis of complex chromatin modification patterns based on recombinant, uniformly modified chromatin templates in combination with quantitative SILAC-based mass spectrometry analysis. In this manner, we defined the interactome of the H3K4me3 and H3K9me3 chromatin marks. Surprisingly, only some factors were also recruited to corresponding histone N-terminal peptides in parallel experiments. Our results set the stage for using chromatin-based affinity approaches to investigate how the histone code is read and translated on a global scale.

EXPERIMENTAL PROCEDURES

Cell Culture, Labeling, and Nuclear Extract Preparation—HeLa S3 cells were grown in lysine- and arginine-deficient Dulbecco’s modified Eagle’s medium supplemented with 10% dialyzed fetal bovine serum (PAA, Pasching, Austria). One cell population was supplemented with normal isotope containing L-lysine and L-arginine (Sigma, Munich, Germany) and another with heavy isotope labeled 13C6-lysine and 13C6-15N-arginine (Euroisper, Top, Saint-Aubin Cedex, France) generating mass shifts of +6 and +10 Da, respectively. Cells were grown for at least six passages at smaller volumes and then expanded to 2 l in spinner flasks (0.5–1.0 × 10^6 cells/ml) (16). The cells were then transferred to a 5 l fermenter (Applikon, Schiedam, Netherlands) and grown under standard conditions (2.5–5.0 × 10^6 cells/ml). Harvested cells were used to prepare nuclear extract according to standard procedures (17).

Peptides—Peptides containing the 20 N-terminal amino acids of histone H3 were synthesized in unmodified and modified form using Fmoc (N-(9-fluorenyl)methoxycarbonyl)-based solid-phase synthesis H3unmodified: ARTKQTARKSTGGKAPRKQL; H3K4me3: ARTK(me3)-QTKARSGTGGKAPRKQL; H3K9me3: ARTKQTARM(me3)SGTGKAPRKQL. Peptides contained a C-terminal non-native lysine biotinylated at the ε-amino group for affinity purification reactions or were transformed to thioacetamidophenylesters for native protein ligation (18–20).

Native Protein Ligation—Histone modifications were achieved by native protein ligation using histone H3 (1–20) thioester peptides and recombinant X. laevis histone H3.1–20.A21C as described (21). Reactions were carried out in 100 mM potassium phosphate (pH 7.9), 3 mM guanidine-HCl, 0.5% v/v benzyl mercaptan, 0.5% v/v thiophenol at 25°C with vigorous mixing. Crude reaction mixture was diluted 50-fold into SAU-200 buffer (7 M deionized urea, 200 mM sodium acetate (pH 5.2), 1 mM EDTA, 1 mM dithiothreitol, 200 mM NaCl), applied to a 5 ml Hi-Trap SP-Sepharose high performance cation exchange column (GE Healthcare, Munich, Germany), and eluted with a linear NaCl gradient from 200 to 600 mM in 10 column volumes. Protein was dialyzed extensively against 2 mM dithiothreitol at 4°C, lyophilized and stored at –80°C. Routinely we set up ligation reactions containing 27 mg histone (2 μmol), 23 mg thioacetamidophenylester histone H3 peptide (10 μmol) in 10 ml reaction volume. After purification on average 10 mg ligated histone H3 was obtained (0.6 μmol). Purity and identity of thioester peptides and ligated proteins was confirmed by analytical high-performance liquid chromatography, mass spectrometry, and SDS-PAGE (see supplemental Fig. S1).

Recombinant Chromatin—Recombinant chromatin was prepared essentially as described (22). Briefly, recombinant wild type Xenopus laevis histones were expressed in Escherichia coli and purified as described (23). Assembly of histone octamers containing C-terminal modified and unmodified histone H3 as well as nucleosome array reconstitution was performed by salt dialysis on biotinylated 12 × 200 × 601 DNA template as described (23, 24). Quality of chromatin reconstitution was monitored by native agarose gel electrophoresis, MNase digest, and analytical ultracentrifugation (see supplemental Fig. S2).

Peptide and Chromatin Affinity Purifications—Affinity purifications were performed essentially as described using two separate preparations of nuclear extract (22). Each experiment was performed in “forward” (light extract, unmodified chromatin and peptide; heavy extract, modified chromatin and peptide) and “reverse” (light extract, modified chromatin and peptide; heavy extract, unmodified chromatin and peptide) label swap affinity purification. For peptide affinity purifications, 40 μl prewashed streptavidin coated paramagnetic beads (Pierce, Rockford, IL) were saturated with 10 μg biotinylated histone peptide overnight at 4°C. A 0.5 ml aliquot of precleared HeLa S3 nuclear extract (light or heavy isotope labeled) was incubated with the peptide-bound paramagnetic beads for 4 h while rotating. Beads
were washed three times with 1 ml of PD150 buffer (20 mm HEPES pH 7.9, 150 mm NaCl, 0.1% Triton X-100, 5% glycerol). Beads from parallel affinity purification reactions using unmodified and modified peptides were mixed (25) and bound proteins were eluted with LDS sample buffer (Invitrogen, Carlsbad, CA). Chromatin affinity purifications were performed according using 50 μg chromatin and 200 μl paramagnetic beads. To improve SDS-PAGE resolution, eluates of chromatin affinity purification reactions were incubated with 1 kU benzonase (Calbiochem, San Diego, CA) nuclease for 1 h at 37 °C.

Western Blotting— Primary antibodies used were: αH3K4me3 (1:2,000, Abcam, Cambridge, UK), αH3K9me3 (1:1,000, Millipore, Billerica, MA), αFLAG (1:1,000, Sigma, Munich, Germany), and αSMCHD1 (1:1,000, Abcam, Cambridge, UK).

LC-MS/MS— Eluted proteins were separated on 4–12% gradient SDS-PAGE gels (Invitrogen, Carlsbad, CA) and stained with Colloidal Coomassie Blue. Each gel lane was cut into 23 equal gel slices and proteins therein were in-gel digested with trypsin (Promega, Madison, WI) as described (26). Tryptic peptides from each gel slice were extracted and analyzed by nanoflow HPLC (Agilent, Boeblingen, Germany) as described (26). Tryptic peptides from each gel slice were extracted and analyzed by nanoflow HPLC (Agilent, Boeblingen, Germany) as described (26). Tryptic peptides from each gel slice were extracted and analyzed by nanoflow HPLC (Agilent, Boeblingen, Germany) as described (26). Tryptic peptides from each gel slice were extracted and analyzed by nanoflow HPLC (Agilent, Boeblingen, Germany) as described (26). Tryptic peptides from each gel slice were extracted and analyzed by nanoflow HPLC (Agilent, Boeblingen, Germany) as described (26). 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and deposited onto 5′ biotinylated 12 × 200 × 601 DNA template, which contains strong nucleosome positioning potential, using the salt dialysis method (23, 24). Material assembled with unmodified H3, H3K4me3, and H3K9me3 was analyzed by SDS-PAGE and via MNase digest confirming similar histone content and nucleosome density (supplementary Fig. S2). Additional biophysical analysis using analytical ultracentrifugation and atomic force microscopy verified identical hydrodynamic and structural behavior of the different chromatin templates (data not shown).

Biotinylated, reconstituted oligonucleosomes or synthetic peptides unmodified or containing the H3K4me3 or H3K9me3 modifications were immobilized on magnetic streptavidin beads and incubated with nuclear extract prepared from HeLa S3 cells grown under SILAC conditions. We used normal isotope containing L-lysine and L-arginine for preparing “light” extracts and heavy isotope labeled 13C6-lysine (Lys6) and 13C6-15N4-arginine (Arg10) for generating “heavy” extracts. Extracts were analyzed in trial experiments to verify high synchronicity of “light” and “heavy” material (i.e. both extracts contain the same proteins and in the same amounts but only differ in the labeling of the proteins, supplemental Fig. S3). In forward experiments unmodified chromatin or peptide templates were incubated with “light” extracts, whereas corresponding H3K4me3 or H3K9me3 matrices were incubated with “heavy” extracts. In reverse experiments this order was swapped (label swap experiment).

Western blot analysis of the chromatin templates before and after incubation with extract verified the identity of the modifications (Fig. 1B). Importantly, no loss of H3K4me3 or H3K9me3 after exposure to nuclear extracts was observed. Similarly, mass spectrometric analysis verified identity of the histone H3 N-terminal peptides (data not shown).

Proteins specifically retained on the different matrices were recovered from the beads. Eluates were mixed in 1:1 ratio, run on SDS-PAGE gels and the separated proteins were trypsinized. The tryptic peptides were then analyzed by mass spectrometry and quantitative ratios for heavy and light SILAC pairs were determined (Fig. 1C). To minimize random variation, experiments were repeated twice (biological replicates) for each set-up, i.e. each combination of unmodified versus H3K4me3 or H3K9me3 was run twice in the forward and twice in the reverse direction. Each reaction was analyzed three times (technical replicates) on the mass spectrometer to maximize the probability of faithful identification of all factors in the samples.

H3K4me3 and H3K9me3 Interactomes—We analyzed a total of ca. 2000 factors in each of the affinity purification experiments out of approximately 5000 proteins present in the HeLa S3 nuclear extract (data not shown). These include proteins specifically and unspecifically enriched on the streptavidin beads as well as the chromatin or peptide containing matrices (Fig. 2). By comparing unmodified and modified templates we detected factors enriched (i.e. preferentially binding modified versus unmodified) or excluded (i.e. preferentially binding unmodified versus modified) by the H3K4me3 and H3K9me3 modifications for chromatin as well as peptide-containing matrices to different degrees beyond this background. Applying a relatively stringent threshold of fourfold change, 59 of these were found enriched on H3K4me3 chromatin of which 32 were represented in forward and reverse experiments (Table I, Fig. 2A). For the H3K4me3 peptide we found 71 factors enriched with 60 of these represented in forward and reverse experiments; 22 factors were excluded from this matrix with eleven found in forward and reverse experiments (Fig. 2B). For the H3K9me3 chromatin affinity purification we found 39 factors enriched by this modification of which 21 were represented in forward and reverse experiments; nine factors were found excluded from this matrix with two represented in forward and reverse experiments.
Fig. 2. Identification of factors recruited by H3K4me3 or H3K9me3 using recombinant, uniformly modified chromatin or histone tail peptide affinity purification and SILAC-MS. A, H3K4me3 chromatin. B, H3K4me3 histone tail peptide. C, H3K9me3 chromatin. D, H3K9me3 histone tail peptide. Upper panels: Scatter plots representing normalized ratios of identified and quantified proteins and total summed peptide intensities. Proteins identified in forward and reverse set-up of the experiment with ratio change above four are colored in red; those only identified in forward or reverse set-up of the experiment but with a ratio change above four are colored in purple. Factors with enrichment or exclusion ratios below four are colored in blue. Lower panels: Scatter plots representing normalized ratios of identified and quantified proteins in both, forward and reverse experiments. Proteins with ratio change above four are shown in red; those with enrichment or exclusion ratios below four are colored in blue. For details on the identified proteins see Tables I and II. In all plots proteins showing opposite ratios between forward and reverse experimental set-up (and therefore potential false positives) are omitted.
Table 1
Factors found enriched or excluded on H3K4me3 chromatin and/or peptide templates from HeLa S3 nuclear extracts. Factors in italics were identified only in forward or reverse set-up of the experiments. Asterisks mark factors that were identified in forward and reverse set-up of the experiment with either peptide or chromatin templates, but were only found in forward or reverse set-up of the experiment using the other matrix. Factors highlighted in red were verified as direct H3K4me3 binding proteins in independent biochemical experiments using recombinant proteins (please refer to the indicated references for study details). Factors highlighted in blue were also found in a recent SILAC MS study using modified mononucleosomes for affinity purification (46). Factors highlighted in green were also found in a recent SILAC MS study using histone tail peptides for affinity purification (6). Factors highlighted in brown were found overlapping in two recent SILAC MS studies, in histone tail peptide and mononucleosome based experiments (6, 46). Verified interaction factors that were not identified in a recent study using modified mononucleosomes for affinity purification are marked with § (46). Verified interaction factors that were not identified in a recent study using histone tail peptides for affinity purification are marked with # (6).

| IPI number, gene name | Domains, motifs and regions of similarity | Functional relevance |
|-----------------------|------------------------------------------|----------------------|
| **H3K4me3**           |                                          |                      |
| Enriched with chromatin and peptide |                      |                      |
| IPI00944951: TAF6      | DUF1546, TAF                           | Transcription factor TFIID subunit |
| IPI00413755: TAF4      | TAFH, TAF4                              | Transcription factor TFIID subunit |
| IPI00550655: SPIN1     | Spin-Sety                              | Cell cycle regulation |
| IPI00663434: PHF23*    | PHD                                     |                      |
| IPI0018108: TAF13*     | TFIIID-18kDa                            | Transcription factor TFIID subunit |
| IPI00181111: TAF7      | TAFI55_N                               | Transcription factor TFIID subunit |
| IPI0004350: GTF2A1*    | TFIIA                                   | Transcriptional activation |
| IPI00853240: TAF3* (29) | Bromo TP                              | Transcription factor TFIID subunit |
| IPI00480187: PHF8 (30) | JmjC, PHD                               |                      |
| IPI00872314: EMSY      | ENT                                     | Transcriptional repression |
| IPI00297250: PHF12     | PHD                                     | Transcriptional repression |
| IPI00299825: TAF5      | TFIIID_90kDa, WD40                      | Transcription factor TFIID subunit |
| IPI00645793: TAF1      | Bromodomain, TBP-binding                | Transcription factor TFIID subunit |
| IPI0007589: SIN3B      | HDAC Interact, PAH                      | Transcription factor TFIID subunit |
| IPI0018110: TAF11      | TAFI28                                  | Transcription factor TFIID subunit |
| IPI00018041: MAGEA3*   | MAGE                                    |                      |

| Enriched with chromatin |                      |                      |
| IPI00297851: CHD1 (57)  | Chromo, Helicase C, SNF2 N             | ATP-dependant helicase |
| IPI00006651: SUPPT7L    | Bromo TP                               | STAGA complex subunit |
| IPI00010365: USP22      | UCH, UBp zink finger                   | Ubiquitin carboxyl-terminal hydrolase |
| IPI00550119: SEPT6      | Septin                                 | Involved in cytokinesis |
| IPI0046738: FAM48A      |                                       | Gastrulation regulation |
| IPI00005769: FANCG      |                                       | DNA repair |
| IPI00009355: PARP12     | PARP, CCCH zink finger                 | Poly [ADP-ribose] polymerase |
| IPI00009290: FANCF      |                                       | DNA repair |
| IPI00044945: BAX        | Bcl-2                                  | Apoptosis regulation |
| IPI00014843: LRRC16A    |                                       | Actin filament elongation |
| IPI00550523: ATL3       | GBP                                    |                      |
| IPI00016342: RAB7A      | Ras                                    | Endocytic transport |
| IPI00025202: FMNL1      | Drf_FH3, Drf_GBD, FH2                  | Cell mobility and survival of macrophages |
| IPI00004859: BLM        | BDHCT, DEAD, Helicase_C, HRDC, RQC     | DNA replication and repair helicase |
| IPI00419575: C7orf20    | DUF410                                 |                      |
| IPI00022462: TFRC       | PA, TFR dimer                          | Iron uptake |
| IPI00009898: SLC7A5     | AA permease                            | Amino acid transport |
| IPI00300096: RAB35      | Ras                                    | Ras-related GTPase   |
| IPI00175193: KIF4B      | Kinesin                                | Cytokinesis |
| IPI00291510: IMPDH2     | CBS, IMPDH                             | Regulation of nucleotide biosynthesis |
| IPI00031960: POLR1A     | RNA_pol_Rpb1                           | rRNA synthesis |
| IPI00023608: FANCC      | Fancon C                               | DNA repair |
| IPI00152503: DTX3L      | C3HC4 zink finger                      | Ubiquitin ligase |
| IPI00014977: MCM9       | MCM                                    |                      |
| IPI00069430: GTF2A1     | TFIIA                                  | Transcriptional activation |
| IPI00478737: RFWD3      | WD40, C3HC4 zink finger                |                      |
| IPI00008137: ZNF25S     | BTB, C2H2 zink finger                  | Transcriptional regulation |
| IPI00032496: MCM8       | MCM                                    | Cell proliferation control |
| IPI00022055: PCAF       | Acetyltransfer 1, Bromo, PCAF_N       | Transcriptional activating HAT |
| IPI00915456: DAXX       | Daxx                                   | Apoptosis regulation |
| IPI00885015: FANCL      | WD-3                                   | DNA repair ubiquitin ligase |
| IPI00013885: CASP14 | Peptidase C14 | Epidermal differentiation |
|----------------------|--------------|--------------------------|
| IPI00022831: TBP     | TBP          | Transcriptional activation |
| IPI00016930: ING2^a^ (32) | PHD         | HAT and HDAC regulation |
| IPI00166009: KDM2A    | F-box, JmjC, CXXC zink finger | Histone lysine demethylase |
| IPI00171123: GATA1    | GATA         | Transcriptional activation |
| IPI00373869: C17orf49 |              |                          |
| IPI00607645: SUDS3    | Sds3         | Transcriptional repression |
| IPI00099385: ING1     | PHD          | Transcriptional repression |
| IPI00031653: BRMS1L   | Sds3         | Transcriptional repression |
| IPI00170596: SIN3A    |              | Transcriptional repression |
| IPI00018510: SPIN2A   | Spin-Stry    | Cell cycle regulation    |
| IPI00785110: BPTF     | Bromodomain, PHD | Transcriptional activation |
| IPI0002806: TAF12     | TFIID-20kDa  | Transcriptional activation |
| IPI00872208: TNRC18   | BAH          | Transcriptional activation |
| IPI000116: TAF9       | TFIID-31kDa  | Transcriptional regulation |
| IPI00941164: TAF4B    | TAF4, TAFH   | Transcriptional repression |
| IPI00021363: KDM5A    | ARID, JmjN, PHD, PLU-1, C5HC2 zink finger | Histone lysine demethylase |
| IPI00002220: SAP130   |              | Transcriptional repression |
| IPI00022019: SAP30    |              | Transcriptional repression |
| IPI00065313: TAF8     | Bromo TP, TAF8 C | Transcriptional activation |
| IPI00030364: TAF10    | TFIID-30kDa  | Transcriptional repression |
| IPI00642105: TAF9B    | TFIID-31kDa  | Transcriptional repression |
| IPI00328928: ARID4B   | ARID, RBB1NT | Transcriptional repression |
| IPI00607648: BRMS1    | Sds3         | Transcriptional repression |
| IPI00002831: SAP30L   |              | Transcriptional repression |
| IPI00455982: HMGXB4   | HMG box      | Transcriptional repression |
| IPI00171309: PHF13    | PHD          | Transcriptional repression |
| IPI00061680: CCDC10   | DUF1325      | Transcriptional repression |
| IPI00008054: BRPF3    | Bromodomain, PHD, EPL1, PWWP | Transcriptional repression |
| IPI00328144: TAF2     | Peptidase M1 | Transcriptional repression |
| IPI00641026: CXXC1    | PHD, CXXC zink finger | Transcriptional repression |
| IPI00639887: FAM60A   |              | Transcriptional repression |
| IPI00398103:          |              | Transcriptional repression |
| IPI00847793: DCD      |              | Pathogen defence         |
| IPI00654744: SYNGR2   | MARVEL       | Transcriptional repression |
| IPI00028109: DPY30    | Dpy-30       | Transcriptional repression |
| IPI00914930: ANKRD11  | Ank          | Transcriptional repression |
| IPI0087617: PHF2      | JmjC, PHD    | Transcriptional repression |
| IPI00550968: MORF4L1  | MRG          | Transcriptional repression |
| IPI00782935: INGS      | PHD          | Transcriptional repression |
| IPI00922181: MCM2     | MCM          | Transcriptional repression |
| IPI00743143: ING4     | PHD          | Transcriptional repression |
| IPI00005492: WDR5 (33) | WD40     | Transcriptional repression |
| IPI00006029: FOXX2    | FHA, Fox head | Transcriptional repression |
| IPI00180764: MYST2    | MOZ SAS, C2HC zink finger | Transcriptional repression |
| IPI00292376: GMIP     | C11, RhoGAP  | Transcriptional repression |

**Enriched with peptide**

| IPI00013885: CASP14 | Peptidase C14 | Epidermal differentiation |
|----------------------|--------------|--------------------------|
| IPI00022831: TBP     | TBP          | Transcriptional activation |
| IPI00016930: ING2^a^ (32) | PHD         | HAT and HDAC regulation |
| IPI00166009: KDM2A    | F-box, JmjC, CXXC zink finger | Histone lysine demethylase |
| IPI00171123: GATA1    | GATA         | Transcriptional activation |
| IPI00373869: C17orf49 |              |                          |
| IPI00607645: SUDS3    | Sds3         | Transcriptional repression |
| IPI00099385: ING1     | PHD          | Transcriptional repression |
| IPI00031653: BRMS1L   | Sds3         | Transcriptional repression |
| IPI00170596: SIN3A    |              | Transcriptional repression |
| IPI00018510: SPIN2A   | Spin-Stry    | Cell cycle regulation    |
| IPI00785110: BPTF     | Bromodomain, PHD | Transcriptional activation |
| IPI0002806: TAF12     | TFIID-20kDa  | Transcriptional activation |
| IPI00872208: TNRC18   | BAH          | Transcriptional activation |
| IPI0002993: TAF9      | TFIID-31kDa  | Transcriptional regulation |
| IPI00941164: TAF4B    | TAF4, TAFH   | Transcriptional regulation |
| IPI00021363: KDM5A    | ARID, JmjN, PHD, PLU-1, C5HC2 zink finger | Histone lysine demethylase |
| IPI00002220: SAP130   |              | Transcriptional repression |
| IPI00022019: SAP30    |              | Transcriptional repression |
| IPI00065313: TAF8     | Bromo TP, TAF8 C | Transcriptional activation |
| IPI00030364: TAF10    | TFIID-30kDa  | Transcriptional regulation |
| IPI00642105: TAF9B    | TFIID-31kDa  | Transcriptional repression |
| IPI00328928: ARID4B   | ARID, RBB1NT | Transcriptional repression |
| IPI00607648: BRMS1    | Sds3         | Transcriptional repression |
| IPI00002831: SAP30L   |              | Transcriptional repression |
| IPI00455982: HMGXB4   | HMG box      | Transcriptional repression |
| IPI00171309: PHF13    | PHD          | Transcriptional repression |
| IPI00061680: CCDC10   | DUF1325      | Transcriptional repression |
| IPI00008054: BRPF3    | Bromodomain, PHD, EPL1, PWWP | Transcriptional repression |
| IPI00328144: TAF2     | Peptidase M1 | Transcriptional repression |
| IPI00641026: CXXC1    | PHD, CXXC zink finger | Transcriptional repression |
| IPI00639887: FAM60A   |              | Transcriptional repression |
| IPI00398103:          |              | Transcriptional repression |
| IPI00847793: DCD      |              | Pathogen defence         |
| IPI00654744: SYNGR2   | MARVEL       | Transcriptional repression |
| IPI00028109: DPY30    | Dpy-30       | Transcriptional repression |
| IPI00914930: ANKRD11  | Ank          | Transcriptional repression |
| IPI0087617: PHF2      | JmjC, PHD    | Transcriptional repression |
| IPI00550968: MORF4L1  | MRG          | Transcriptional repression |
| IPI00782935: INGS      | PHD          | Transcriptional repression |
| IPI00922181: MCM2     | MCM          | Transcriptional repression |
| IPI00743143: ING4     | PHD          | Transcriptional repression |
| IPI00005492: WDR5 (33) | WD40     | Transcriptional repression |
| IPI00006029: FOXX2    | FHA, Fox head | Transcriptional repression |
| IPI00180764: MYST2    | MOZ SAS, C2HC zink finger | Transcriptional repression |
| IPI00292376: GMIP     | C11, RhoGAP  | Transcriptional repression |
reverse experiments (Table II, Fig. 2C). With the H3K9me3 histone N-terminal tail peptide we found 53 factors enriched with 40 of these found in forward and reverse experiments; four factors were excluded by this modification on the peptide of which one was represented in forward and reverse experiments (Fig. 2D).

### TABLE I—continued

| Accession | Name | Description |
|-----------|------|-------------|
| IPI000412787: ARL5A | Arf | Transcriptional regulation |
| IPI00004344: AFF4 | AF-4 | Histone lysine demethylase |
| IPI00847436: KDM5B | ARID, JmjN, PHD, PLU-1, CSHC2 zink finger | Transcriptional regulation |
| IPI00060777: PHF16 | EPL1, PHD | Transcriptional regulation |
| IPI00396967: FHIL2 | LIM | Transcriptional regulation |
| IPI0009373: EAF6 | NuA4 | Apoptosis regulation |
| IPI00306794: CARD6 | CARD | Transcriptional regulation |
| IPI00658062: ZNF131 | BTB, C2H2 zink finger | Transcriptional regulation |

#### Excluded from chromatin and peptide

| Accession | Name | Description |
|-----------|------|-------------|
| IPI00472782: PHF14 | PHD | Nucleoside biosynthesis |
| IPI000604590: NME1-NME2 | NDK | |

#### Excluded from chromatin

| Accession | Name | Description |
|-----------|------|-------------|
| IPI00797279: UHRF1 | PHD, ubiquitin, YDG SRA | Transcriptional regulation, ubiquitin ligase |
| IPI00296772: GGA2 | Alpha adaptin C2, GAT, VHS | Protein sorting |
| IPI00003965: USP7 | MATH, UCH | Ubiquitin carboxyl-terminal hydrolase |
| IPI00898444: CAMK2G | CaMKIIID, Protein kinase | Potentiation and neurotransmitter release |
| IPI0031519: DNMT1 | BAH, DMAP binding, DNA methylase, CXXC zink finger | CpG methylation, transcriptional repression |
| IPI00167031: IL34 | | Immune responce |
| IPI0074954: | | |

#### Excluded from peptide

| Accession | Name | Description |
|-----------|------|-------------|
| IPI00024719: HAT1 | Hat1 N | Non-nucleosomal HAT |
| IPI00879166: MTA1 | BAH, ELM2, GATA, Myb DNA binding | Transcriptional regulation |
| IPI00439194: MBD3 | MBD | Transcriptional repression, meCpG binding |
| IPI00434623: MBD2 | MBD | Transcriptional repression, meCpG binding |
| IPI00103554: GATAD2B | GATA | Transcriptional repression |
| IPI00171798: MTA2 | BAH, ELM2, GATA, Myb DNA binding | Transcriptional regulation |
| IPI00018198: HAUS2 | | |
| IPI00744834: STX5 | SNARE, Syntaxin | Vesicular transport |
| IPI00062037: DYNL2 | Dynein light | Intracellular transport and motility |
| IPI00020557: LRP1 | EGF, EGF CA, Ldl recept A/B | Lipid metabolism |
| IPI00844507: C1orf174 | | |
| IPI00143767: RAB31 | | |
| IPI0026520: TMASF1 | L6 membrane | |
| IPI00465222: CHD3 | Chromo, PHD, SNF2 N, Helicase C, CHDCT2, CHDNT, DUF1086/1087 | Transcriptional regulation, helicase |
| IPI00478128: GATAD2A | GATA | Transcriptional repression |
| IPI00828172: FAM114A2 | DUF719 | |
| IPI00465028: TPI1 | TIM | Core metabolism |
| IPI00852806: | Sec15 | Vesicular transport |
| IPI00414985: C13orf27 | | |
| IPI0008986: SLC7A5 | AA permease | Amino acid transport |

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*Only factors with a four-fold enrichment over the corresponding unmodified templates are given. Table entries are ranked according to the fold enrichment.

* Proteins are identified via the International Protein Index (IPI) number. Only one of in may cases several protein names are listed.

* Protein domains and motifs were derived from Pfam (56).

* Functional relevance according to STRING (54).
TABLE II
Factors found enriched or excluded on H3K9me3 chromatin and/or peptide templates from HeLa S3 nuclear extracts. a, b, c, d Factors in italics were identified only in forward or reverse set-up of the experiments. Asterisks mark factors that were identified in forward and reverse set-up of the experiment with either peptide or chromatin templates, but were only found in forward or reverse set-up of the experiment using the other matrix. Factors highlighted in red were verified as direct H3K9me3 binding proteins in independent biochemical experiments using recombinant proteins (please refer to the indicated references for study details). Factors highlighted in blue were also found in a recent SILAC MS study using modified mononucleosomes for affinity purification (46). Factors highlighted in green were also found in a recent SILAC MS study using histone tail peptides for affinity purification (6). Factors highlighted in brown were found overlapping in two recent SILAC MS studies, in histone tail peptide and mononucleosome based experiments (6, 46). Verified interaction factors that were not identified in a recent study using modified mononucleosomes for affinity purification are marked with § (46). Verified interaction factors that were not identified in a recent study using histone tail peptides for affinity purification are marked with # (6).

| IPI number, gene name | Domains, motifs and regions of similarity | Functional relevance |
|----------------------|------------------------------------------|---------------------|
| **H3K9me3**          |                                          |                     |
| **Enriched with chromatin and peptide** |                                          |                     |
| IPI00890837: SMCHD1  | HATPase, SMC hinge                       |                     |
| IPI00022215: ADNP    | Homeobox, C2H2 zink finger               | Transcriptional regulation |
| IPI00878669: CBX1 (34, 58) | Chromo, Chromo shadow                  | Epigenetic repression |
| IPI00297579: CBX3 (34, 58) | Chromo, Chromo shadow                  | Epigenetic repression |
| IPI00204662: CBX5 (34, 58) | Chromo, Chromo shadow                  | Epigenetic repression |
| IPI00845355: ATRX    | Helicase C, SNF2 N                       | Transcriptional regulation |
| IPI00410720: POGZ    | CenB DNA binding, DDE, C2H2 zink finger |                     |
| IPI00011857: CHAF1B  | WD40                                      | Chromatin assembly   |
| IPI00023177: CHAF1A  |                                          |                     |
| IPI00797279: UHRF1 (35) | PHD, ubiquitin, YDG SRA            | Transcriptional regulation, ubiquitin ligase |
| IPI00641109: ZMY3M   | FCS zink figer                           |                     |
| IPI00477949: ZMY4    | FCS zink figer                           |                     |
| IPI00464861: UHRF2   | PHD, ubiquitin, YDG SRA, C3HC4 zink finger | Transcriptional regulation, ubiquitin ligase |
| IPI00436632: NIPBL   |                                          | Chromatid cohesion   |
| IPI00064212: ZNF828  | C2H2 zink figer                          |                     |
| IPI000915456: DAXX   | Daxx                                     | Apoptosis regulation |
| IPI00479789: C1orf103|                                          |                     |
| IPI00294603: ZMY2*   | FCS zink figer                           | Transcriptional regulation |
| IPI00000656: KIAA0892| Cohesin load                            |                     |
| IPI00329820: ACTL8   | Actin                                    |                     |
| **Enriched with chromatin** |                                          |                     |
| IPI00025753: DSG1   | Cadherin, Cadherin C                     |                     |
| IPI00010948: TRIM26  | SPRY, B-box zink finger, C3HC4 zink fnger |                     |
| IPI00410287: PRKAAA1| Protein kinase                           |                     |
| IPI00913848: FERMT2  | Ferm M, PH                               |                     |
| IPI00852685: DIAPH1  | Dfr DAD/FH1/FH3/GBD, FH2                 |                     |
| IPI00397904: NUP93   | Nic96                                    |                     |
| IPI00402657: RPAP1   | RPAP1 C/N                                |                     |
| IPI00219518: ARL1    | Arf                                      |                     |
| IPI00397801: FLG2    | Efhand, Flaggrin, S100, SVS QK           |                     |
| IPI00947285:         |                                          |                     |
| IPI00004317: POLG    | DNA Pol A                                | Mitochondrial DNA replication |
| IPI00216099: DSC1    | Cadherin, Cadherin Pro                   |                     |
| IPI00026256: FLG     | Flaggrin, S100                          |                     |
| IPI00103242: POF1B   |                                          |                     |
| IPI00878849: P2RX5   | P2X receptor                             |                     |
| IPI00021536: CALM5L  | Efhand                                   |                     |
| IPI00071509: PKP1    | Arm                                      |                     |
| IPI00099949: PSMF1   | P31 Prot reg                             |                     |
| IPI0009777: ZNF581   | C2H2 zink finger                         |                     |
| **Enriched with peptide** |                                          |                     |
| IPI00438229: TRIM28  | PHD, B-box zink finge, C3HC4 zink finge  |                     |
| IPI00402209: ADNP2   |                                          |                     |
| IPI00855833: PRR12   |                                          |                     |
| IPI00396015: ACACA   |                                          |                     |
| IPI00010252: TRIM33  |                                          |                     |

*Chromatin Modification Interactome*
The different data sets contain factors that have been shown to directly interact with H3K4me3 or H3K9me3 in independent biochemical experiments, thereby validating our approach. TAF3 (29), PHF8 (30), CHD1 (31), ING2 (32), and WDR5 (33) are known H3K4me3 interacting proteins. The HP1 isoform proteins CBX1, CBX3, CBX5 (34), as well as UHRF1 (35), CDYL1 (36), and MPHOS8 (37) have been shown to bind H3K9me3. Besides direct interaction partners, the data sets also include proteins that are known to be indirectly recruited to the chromatin marks. For example, TFIID components (e.g., TAF1–2, 4–13) are recruited to H3K4me3 via TAF3 (29). POGZ is bound to H3K9me3 via CBX5 (38). Known or predicted functional interactions between the identified enriched proteins are shown in supplemental Fig. S4. Additional work will be necessary to investigate which of the newly identified components interact directly or indirectly with the chromatin marks.

Overall, the data present a significant expansion of the repertoire of factors that are recruited to H3K4me3 or H3K9me3.

### TABLE II—continued

| Gene ID       | Function                          | Domain/Motif                  | Chromatin Interaction                  |
|---------------|-----------------------------------|-------------------------------|----------------------------------------|
| IPI00936015:  | ACC central, Biotin carb C, Biotin lipoil, Carboxytrans, |                               | Core metabolism                        |
| IPI00293963:  | C2H2 zink finger                  |                               | Calcium binding                       |
| IPI00514648:  | BRK, Bromodomain, Helicase C, HAS, QLO, SFN2 N |                               | Immune response                        |
| IPI00642271:  | UAA                              |                               | Glutathione conjugation                |
| IPI00639924:  | C19orf68                         |                               | Immune response                        |
| IPI00003965:  | MATH, UCH                        |                               | Proteasome regulation                 |
| IPI00019520:  | KRAB, SCAN, C2H2 zink finger      |                               | Transcriptional regulation             |
| IPI00025494:  | APC2, Cullin                      |                               | Ubiquitin carboxy-terminal hydrolase   |
| IPI00024411:  | Syndecan                         |                               | Intra cellular transport and motility  |
| IPI00419402:  | KRAB, C2H2 zink finger            |                               | Cell cycle regulation                  |
| IPI00302755:  | KRAB, C2H2 zink finger            |                               | Cell contact                          |
| IPI00306446:  | SCAN, C2H2 zink finger            |                               | Transcriptional regulation             |
| IPI00410039:  |                                 |                               | Transcriptional regulation             |
| IPI00008531:  | ELM2, Myb DNA binding             |                               | Epidermal differentiation              |
| IPI00019329:  | Dynein light                      |                               |                                       |
| IPI00514648:  | SMARCA2                          |                               |                                       |
| IPI00642271:  | SLC35B2                          |                               |                                       |
| IPI00639924:  | C19orf68                         |                               |                                       |
| IPI00219757:  | GSTP1                            |                               |                                       |
| IPI00219806:  | APC2, Cullin                      |                               |                                       |
| IPI00003965:  | C2H2 zink finger                  |                               |                                       |
| IPI00019520:  |                                 |                               |                                       |
| IPI00025494:  |                                 |                               |                                       |
| IPI00024411:  |                                 |                               |                                       |
| IPI00419402:  |                                 |                               |                                       |
| IPI00302755:  |                                 |                               |                                       |
| IPI00306446:  |                                 |                               |                                       |
| IPI00410039:  |                                 |                               |                                       |

* Only factors with a four-fold enrichment over the corresponding unmodified templates are given. Table entries are ranked according to the fold enrichment.

* Proteins are identified via the International Protein Index (IPI) number. Only one of in may cases several protein names are listed.

* Protein domains and motifs were derived from Pfam (56).

* Functional relevance according to STRING (54).
H3K9me3 chromatin marks. Nevertheless, it is clear that use of other procedures for preparing extracts as well as different cellular sources (cell type, stage of differentiation) will produce distinct data sets. Applying a lower threshold, the overall number of factors identified using standard Hela S3 nuclear extract is much larger (see supplemental Tables S1–S4 for raw data). However, we feel that a fourfold change presents a robust signal and therefore focused for further analysis on this data set. Analysis of the data using values of significance (“B”) as calculated by the MaxQuant software with a cutoff of 0.05 is shown in supplemental Fig. S5 (39).

We ascribe the fact that around 36% of all factors specifically identified in this study reveal high enrichment or exclusion ratios only in forward or reverse experiments but not in both to the high threshold level of fourfold that we set for the analysis. In fact, detailed examination of the proteins only enriched or excluded in the forward or reverse set-up of the experiment showed that these are largely factors that are more difficult to identify (i.e. these have a lower summed peptide total intensity; see purple colored hits in Fig. 2). Low abundance in the extract, small protein size yielding few peptides, and peptide hydrophobicity might contribute to this phenomenon. Also, batch to batch variability of extracts might play a role in factors only identified in forward or reverse experiments. We tried to minimize this possibility by carefully matching the different extracts used (supplemental Fig. S3). Last, false negative identification (i.e. a factor was by chance not detected in forward or reverse experiment) might be a relevant factor in the analysis. Although proteins identified by both forward and reverse experimental set-up present the most stringent hits, proteins that are found in forward or reverse experiments nevertheless result from averages of four independent experiments (two times forward and two times reverse, see above). A number of factors were also represented only in forward or only in reverse set-up of the experiment using either chromatin or peptide templates, but were identified in both directions of the experiment using the other matrix (annotated with asterisk in Tables I and II). We think that these are therefore meaningful candidates and included them in our further analysis.

Fig. 2 also shows that there is general good agreement between proteins identified in forward and reverse experiments. Interestingly, more factors are enriched than excluded by the H3K4me3 and H3K9me3 histone modifications both, in the context of chromatin as well as peptides. While our type of analysis excludes factors that bind histone H3 irrespective of H3K4me3 or H3K9me3 modifications, we do not think that this observation is caused by a technical problem of the method, but rather presents a “true” finding. The available structural data on different histone modification binding proteins identify highly specialized and in many cases narrow binding pockets where multiple interactions mediate recognition and binding of the PTMs (7). This binding mode likely generates a bigger change in free energy than is achieved by a PTM that is added to a stretch of amino acids recognized by a larger protein surface in the unmodified state. Therefore, the attractive mode might generate more discrimination than the repelling mode. Although additional experiments are necessary to test this hypothesis, we nevertheless note that when going from lower to higher eukaryotes the number and type of histone modifications significantly increases. This phenomenon might reflect the fact that the attractive mode indeed generates more robust signal transduction.

Validation of the Approach—To assess the quality of the new data sets of H3K4me3 and H3K9me3 interacting factors, we choose to independently analyze a set of proteins from each category that had not been analyzed in this context before. We transiently expressed candidate factors in 293 HEK cells and performed peptide or chromatin affinity purification experiments that were analyzed by Western blotting against an engineered FLAG-tag on all factors. As Fig. 3A shows, SPIN1 a factor that is implicated in cell cycle regulation (40) was recovered on the peptide and chromatin H3K4me3 templates, but not on the corresponding unmodified matrices, thereby essentially verifying the results from the SILAC MS analysis. Similarly, we found FANCF, a factor of the Fanconi anemia group (41) specifically enriched on the H3K4me3 chromatin template, but not the corresponding peptide matrix as we had seen in the SILAC MS. Of the H3K9me3 binding factors ADNP, which is a protein containing a neuroactive peptide but that has also nuclear functions (42), and ZMYM3, a Zn-finger protein of unknown function (43) bound specifically to the H3K9me3 peptide and chromatin templates as we had detected in the SILAC MS analysis (Fig. 3B). In contrast, ACTL8, an actin-like protein of unknown function, did not reproduce the findings from the SILAC-MS. We detected the overexpressed protein in comparable amounts bound to the unmodified and H3K9me3 templates. Although this finding might identify a false positive of our screening procedure, we nevertheless point out that ACTL8 contains a PSVLL motif. PxVxL motifs in other proteins have been found to interact with factors of the HP1 type that directly bind H3K9me3 (44). Additional experiments need to find out whether ACTL8 indeed can interact with HP1 and whether overexpression and/or tagging of this factor interfere with its biology. Lastly, we used a specific antiserum against SMCHD1, a factor that might be implicated in the structural maintenance of chromosomes and that was recently found enriched at telomeres (45) to verify that the endogenous, cellular protein is recruited to H3K9me3 (Fig. 3B).

Because the nuclear distribution of H3K4me3 and H3K9me3 are very different, we also analyzed the localization of the new candidate histone methyl-lysine interacting factors in NIH3T3 cells. Here, we found transiently expressed SPIN1 and FANCF diffusely spread in the cell nucleus in a pattern that was reminiscent of H3K4me3 distribution (Fig. 3C). In contrast, transiently expressed ADNP
and ZMYM3 showed enrichment at discrete nuclear foci besides diffuse general localization. As these foci were marked by H3K9me3 and are DNA rich, these represent pericentric heterochromatin. Overall, the results verify five out of six candidates tested as novel interaction partners of H3K4me3 or H3K9me3.

Interestingly, only SPIN1 and ADNP, but neither FANCF, ZMYM3, nor SMCHD1 were found in recent histone tail peptide (6) or mononucleosome (46) H3K4me3 and H3K9me3 affinity purification experiments. Although there is clearly some overlap with the data sets of the peptide based studies (see Tables I and II) the differences of the mononucleosome- and our oligonucleosome-based studies are striking. As the mononucleosome-based data were already analyzed with a very low twofold cutoff of enrichment, these findings might indicate that multiple nucleosomes in the form of arrays provide different and potentially better landing platforms for factors binding to chromatin marks. Although we do not know the basis for the discrepancies in the separate studies, we note that both previously published experiments (6, 46) performed fewer biological replicates, analyzed the data with far lower enrichment cutoffs and did not provide data on the quality of the SILAC extracts used. Additional work is required to resolve potential technical differences in the different studies and to assess the overall quality of the data sets provided.

Comparison of H3K4me3 and H3K9me3 Interactomes—There is generally no correlation of the factors identified with the H3K4me3 and the H3K9me3 histone PTMs, which reflects the different biological contexts that these modifications have been implicated in (Fig. 4A). Only a few proteins were identified in both, the H3K4me3 and H3K9me3 data sets (e.g. DAXX), but then only with chromatin or peptide templates (DAXX was identified only on chromatin with H3K4me3, but on chromatin and peptide templates with H3K9me3). These might represent factors that have a general affinity for tri-methylated lysine residues irrespective of sequence context. Promiscuous binding to (mono- and di-) methyl-lysine marks in different histone sequence environments has for example been observed for proteins containing malignant brain tumor domains (47). Several proteins (e.g. DNMT1, UHRF1) show enrichment with one but exclusion with the other modification. Although these are only represented in either the peptide or chromatin data sets using the stringent fourfold cutoff (e.g. DNMT1 was found enriched not only on the H3K9me3 peptide, but also on H3K9me3 chromatin, but with a 3.7 ratio), such factors could be of high biological interest as they might reflect proteins that mediate binary switches between different totally exclusive chromatin states. On a global level, binary chromatin domains have been described for the activating H3K4me3 and repressing H3K27me3 histone PTMs, where regions that contain both chromatin marks in the pluripotent cell state resolve during differentiation into either of the two states (48).

Interestingly, we find far more factors excluded by H3K4me3 chromatin and peptide templates compared with the H3K9me3 PTM (see Fig. 2). This might indicate that gen-

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**Fig. 3. Verification of H3K4me3 and H3K9me3 interacting factors.** A and B, The indicated proteins containing C-terminal FLAG-tags were transiently expressed in 293T cells. Peptide or chromatin affinity purifications using H3K4me3 (A) or H3K9me3 (B) templates were performed from nuclear extracts and analyzed by Western blot using anti-FLAG antibodies. In case of SMCHD1 extract from untransfected cells was used and the respective affinity purifications were analyzed using anti-SMCHD1 antibodies. Input, 2%. C and D, The indicated proteins containing C-terminal FLAG-tags were transiently expressed in NIH3T3 cells. Immunofluorescence analysis was carried out using anti-FLAG and anti-H3K4me3 (C) or anti-H3K9me3 (D) antibodies. Merged images correspond to the overlay of the two different antibody stainings. DNA was stained with DAPI. Scale bar represents 7.5 μm.
erally more factors can bind to the unmodified very N-terminal region of H3 compared with the H3K9 context. We wonder whether this phenomenon reflects the fact that in higher eukaryotes the default chromatin state is repressive (49). The list of factors excluded by H3K4me3 indeed contains some proteins, which according to gene ontology are implicated in transcriptional repression. Comparison of the interactomes of additional activating and repressive chromatin marks might provide further insights into this behavior.

Comparison of Chromatin and Peptide Affinity Purifications—The total numbers of factors identified by the chromatin or peptide affinity purifications for the H3K4me3 and the H3K9me3 PTMs are quite similar (Fig. 4B). However, the degree of overlap between the proteins recruited by the corresponding chromatin and peptide templates is rather limited. A far larger number of factors are identified only by the peptide-based approach or only by the chromatin-based approach. In light of the fact that both, H3K4me3 and H3K9me3 modifications are very distal from the H3 core domain (the H3 tail region encompasses the first 36 amino acids, ref. 50) and are thereby at the periphery of the nucleosome this finding is rather surprising. A priori one might expect to identify the same factors with both approaches and to potentially recruit and recover more proteins on the chromatin templates. After all, additional interaction surfaces (e.g. DNA, other histone tail and core regions) are available in this native setting. By and large, both approaches might be highly complementary. Future studies will need to tell whether the candidates identified with either approach are more functionally relevant than the other. However, the context of patterns of chromatin modifications especially in the context of DNA methylation as well as in a trans-histone setting can certainly only be investigated by the chromatin-based approach.

For each histone PTM we identified slightly more factors with the peptide templates compared with the chromatin templates. We think this might be a reflection of the potentially higher substitution rate of the peptides on the magnetic streptavidin beads. For immobilization of chromatin representing the same amount of free H3 tails far more beads are needed. Likely, there is steric exclusion on the streptavidin tetramer as well as on the bead surface by the chromatin complex. Also, binding of factors to histone PTMs in the context of chromatin might be subject to repulsive forces by DNA as well as steric hindrance thereby fine-tuning the binding of proteins or multiprotein complexes. Last, we do not know whether the histone tails are fully available on the surface of the nucleosomes in general or under the experimental buffer conditions used (51).

In the case of peptide affinity purifications, a stronger tendency of detecting a protein with one modification as not enriched (1:1 ratio of unmodified to modified templates) and in the other modification as enriched can be seen compared with the chromatin affinity purifications (see Fig. 4A, clouds along the axes). Obviously, the chromatin environment presents the more stringent and more discriminating binding surface.

Fig. 4. Overall evaluation of chromatin and histone tail peptide H3K4me3 and H3K9me3 affinity purifications. Comparison between H3K4me3 and H3K9me3 interactomes in the context of chromatin (A) and peptide (B) affinity purification reactions. Only proteins identified with both modifications are plotted. Chromatin and peptide affinity purifications identify distinct as well as overlapping sets of proteins recruited to H3K4me3 (C) and H3K9me3 (D). Venn diagrams show the number of factors identified with each approach with a fourfold cutoff, both enriched with the specific modification (above line) and excluded by the modification thereby preferentially binding to the unmodified template (below line).
Although the data sets of the H3K9me3 peptide and chromatin affinity purifications as well as the H3K4me3 peptide affinity purifications show a similar overall distribution with relative narrow and focused clustering of the background (blue dots in Fig. 2) as well as distinct highly enriched or excluded factors (red dots in Fig. 2), the results of the H3K4me3 chromatin affinity purification appear different. Here, the background shows an overall wider distribution and only very few factors with high change ratios were identified. Because the results of the H3K4me3 peptide affinity purification are different, this distinction cannot be caused by the histone PTM itself, but must be a consequence of embedding this methyl-lysine mark in a chromatin context. Although we have so far not detected any difference in the biochemical and biophysical behavior of the unmodified, H3K4me3 or H3K9me3 recombinant chromatin preparations, the accessibility of the histone H3 tail might be limited by lysine 4 tri-methylation. This might be a nucleosome restricted effect or be caused by overall chromatin conformational properties (i.e., compaction status). Indeed, single histone modifications affecting the overall behavior of recombinant chromatin templates have been described (52).

Distinct Protein Domains Mediate Recruitment to H3K4me3 and/or H3K9me3—To gain further insight into the recruitment of factors to histone methyl-lysine marks we performed clustering analysis focusing on protein domains (Fig. 5). Prominent chromatin associated protein regions and motifs are highly enriched on the histone methyl-lysine marks. These include several domains directly implicated in methyl-lysine recognition: chromo, PHD, SRA. Also, domains that provide binding interfaces for recruitment of additional proteins are visible e.g., chromoshadow. Other motifs like PxVxL, which has been shown to bind to the chromoshadow domain of HP1 proteins (44, 53), are present in factors presumably indirectly recruited via binding to factors directly interacting with the histone methyl-lysine marks. Common background proteins (ribosomal proteins, tubulins) are present within the middle cutoff group, as are some domains specific for chromatin (helix-loop-helix), peptide (HAT) or both 14-3-3, regardless of the modification used. Although we detect domains that have been implicated in the general context of chromatin (e.g., bromodomains interacting with histone acetylation marks, SNF2 present in nucleosome remodeling factors), DNA (e.g., Zn fingers of various type, PLU-1, ARID, Myb, SANT) or methylated DNA (e.g., methyl-CpG binding domain), these are novel in the context of histone methyl-lysine marks. Common background proteins (ribosomal proteins, tubulins) are present within the middle cutoff group, as are some domains specific for chromatin (helix-loop-helix), peptide (HAT) or both 14-3-3, regardless of the modification used.

Although we detect domains that have been implicated in the general context of chromatin (e.g., bromodomains interacting with histone acetylation marks, SNF2 present in nucleosome remodeling factors), DNA (e.g., Zn fingers of various type, PLU-1, ARID, Myb, SANT) or methylated DNA (e.g., methyl-CpG binding domain), these are novel in the context of histone methyl-lysine marks. It will have to be seen which of these domains are direct histone methyl-lysine mark binding regions and which mediate additional protein–protein interactions in an indirect mode of recruitment. Also, domains that make contact to DNA or other histone regions of the nucleosome might stabilize weaker interactions with sensitivity to and discrimination of the histone PTMs. Analysis for functional interactions using STRING indeed indicates connection groups between several of the identified factors (supplementary Fig. S4) (54). For example, recruitment of the TFIIID complex components has been described via TAF3 binding H3K4me3 (29). Another prominent interaction cluster here is between proteins from the Fanconi anemia group, implicated in the DNA damage response (41). Several of the enriched protein domains might function in the translation of the histone PTMs. This might involve enzymatic activities (e.g., JmjC/ JmjN domains in histone demethylases) as well as regions directly impacting onto chromatin structure. Interestingly, some protein regions are enriched on both, H3K4me3 and H3K9me3 PTMs (e.g., ZnF, PHD, chromo) while being structural and functional parts of different proteins.
Obviously, here the domains have evolved to recognize (tri-)methylated lysine residues in different sequence context, but with high discrimination against the unmodified state exemplifying the multiple use of successful and efficient protein folds and motifs.

CONCLUSIONS

Combination of affinity purification experiments of synthetic peptides representing different histone PTMs either in isolation or in the context of chromatin and quantitative mass spectrometric analysis using SILAC provides a comprehensive set of factors potentially mediating the readout and translation of these marks. Our results show that chromatin affinity purification largely defines a set of factors distinct from what can be found with modified histone tail peptides. We think this might be a general finding as compared to similar analysis recently performed on histone tail peptides (6) or mononucleosomes (46) even show less overlap of factors recruited to different methyl-lysine marks (see also Tables I and II). The chromatin templates provide the advantage of offering additional binding interfaces such as DNA, histone core, other histones and/or other chromatin marks. Thereby, they potentially enable recruitment of multiprotein machineries making several independent contacts to chromatin. Future experiments have to show whether the chromatin data sets indeed contain functionally more relevant factors. In any case, our combined data sets of H3K4me3 and H3K9me3 chromatin and peptide affinity purifications paves the way for additional studies investigating the exact role of the identified proteins in recognition of these methyl-lysine marks as well as in directing chromatin structure and function. Here, combination with array-based technologies that allow screening of direct interactions provides an orthogonal technology (3, 5). Despite characterization of direct interactions with the chromatin marks, emphasis needs to be put onto multiprotein assemblies and interplay of factors that are recruited indirectly. Our global analysis of the protein domains enriched on the chromatin marks as well as the biological connection of factors based on STRING analysis provides a starting point.

Multiple factors might translate individual chromatin marks in different functional settings. In this context, interplay with other histone modifications as well as DNA methylation could manifest an important regulatory mechanism, which has been proposed in the histone code hypothesis (10). Indeed, it is emerging that combinations of chromatin marks mark different domains of chromatin. For example, pericentromeric heterochromatin in higher eukaryotes is enriched in H3 lysine 9 trimethylation (H3K9me3), H4 lysine 20 trimethylation (H4K20me3), H3 lysine 27 monomethylation (H3K27me1) as well as DNA methylation. Linkage to H3 arginine 2 dimethylation (H3R2me2) and H4 arginine 3 dimethylation (H4R3me2) has also been described (55). The use of uniformly modified, recombinant chromatin templates containing different combinations and patterns of chromatin marks for affinity purification experiments will provide an excellent starting point to define the complement of factors that mediate the functional status of such chromatin regions, whose biochemistry has been elusive for a long time.

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This article contains supplemental Figs. S1 to S5, Tables S1 to S4 and Procedures.

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REFERENCES

1. Fischle, W., Wang, Y., and Allis, C. D. (2003) Histone and chromatin cross-talk. Curr. Opin. Cell Biol. 15, 172–183
2. Campos, E. I., and Reinberg, D. (2009) Histones: annotating chromatin. Annu. Rev. Genet 43, 559–599
3. Bua, D. J., Kuo, A. J., Cheung, P., Liu, C. L., Migliori, V., Espejo, A., Casadio, F., Bassi, C., Amati, B., Bedford, M. T., Guccione, E., and Gozani, O. (2009) Epigenome microarray platform for proteome-wide dissection of chromatin-signaling networks. PLoS One 4, e6789
4. Chon, D. W., Wang, Y., Wu, M., Wong, J., Qin, J., and Zhao, Y. (2009) Unbiased proteomic screen for binding proteins to modified lysines on histone H3. Proteomics 9, 2343–2354
5. Liu, H., Galika, M., Iberg, A., Wang, Z., Li, L., Voss, C., Jiang, X., Lajoie, G., Huang, Z., Bedford, M. T., and Li, S. S. (2010) Systematic identification of methyllysine-driven interactions for histone and nonhistone targets. J. Proteome Res. 9, 5827–5836
6. Vermeulen, M., Eberl, H. C., Matarese, F., Marks, H., Derijssen, S., Butter, F., Lee, K. K., Olsen, J. V., Hyman, A. A., Stunnenberg, H. G., and Mann, M. (2010) Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. Cell 142, 967–980
7. Taverna, S. D., Li, H., Ruthenburg, A. J., Allis, C. D., and Patel, D. J. (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat. Struct. Mol. Biol. 14, 1025–1040
8. Adams-Cioaba, M. A., and Min, J. (2009) Structure and function of histone methylation binding proteins. Biochem. Cell Biol. 87, 93–105
9. Fischle, W., Wang, Y., and Allis, C. D. (2003) Binary switches and modification cassettes in histone biology and beyond. Nature 425, 475–479
10. Jenuwein, T., and Allis, C. D. (2001) Translating the histone code. Science 293, 1074–1080
11. Sims, R. J., 3rd, and Reinberg, D. (2008) Is there a code embedded in proteins that is based on post-translational modifications? Nat. Rev. Mol. Cell Biol. 9, 815–820
12. Ruthenburg, A. J., Li, H., Patel, D. J., and Allis, C. D. (2007) Multivalent engagement of chromatin modifications by linked binding modules. Nat. Rev. Mol. Cell Biol. 8, 983–994
13. Fischle, W., Tseng, B. S., Dormann, H. L., Ueberheide, B. M., Garcia, B. A., Shabanowitz, J., Hunt, D. F., Funabiki, H., and Allis, C. D. (2005) Regu-
lation of HP1-chromatin binding by histone H3 methylation and phosphor-ylation. Nature 438, 1116−1122.

14. Liu, H., Lin, D., and Yates, J. R., 3rd (2002) Multidimensional separations for protein/peptide analysis in the post-genomic era. BioTechniques 32, 898, 900, 902 passim.

15. Wilm, M. (2009) Quantitative proteomics in biological research. Proteomics 9, 4590−4605.

16. Ong, S. E., and Mann, M. (2006) A practical recipe for stable isotope labeling by amino acids in cell culture (SILAC). Nat. Protoc. 1, 2650−2660.

17. Dignam, J. D., Martin, P. L., Shastry, B. S., and Roeder, R. G. (1983) Eukaryotic gene transcription with purified components. Methods Enzymol. 101, 582−598.

18. Biancalana, S., Hudson, D., Songster, M. F., and Thompson, S. A. (2001) Fmoc chemistry compatible thio-ligation assembly of proteins. Lett. Peptide Sci. 7, 291−297.

19. Futaki, S., Sogawa, K., Maruyama, J., Asahara, T., Niwa, M., and Hojo, H. (1997) Preparation of peptide tiosteoesters using Fmoc-solid-phase peptide synthesis and its application to the construction of a template-assembled synthetic protein (TASP). Tetrahedron Letters 38, 6237−6240.

20. von Eggelkraut-Gottanka, R., Klose, A., Beck-Sickinger, A. G., and Beyer-Grummt, I. (2010) PHF8 interacts with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development.

21. Schulze, W. X., and Mann, M. (2004) A novel proteomic screen for peptide-protein interactions. J. Biol. Chem. 279, 10756−10764.

22. Shevchenko, A., Tomas, H., Havlis, J., Olsen, J. V., and Mann, M. (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. 1, 2856−2860.

23. 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. Methods 5, 289−296.

24. Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFIID to nucleosomes by chromatin association.

25. Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., and Woodcock, C. L. (2006) Histone H4-K16 acetylation controls chromatin structure and dynamics.

26. Dignam, J. D., Shastry, B. S., and Roeder, R. G. (1983) Preparation of nucleosome core particle from recombinant histones. Methods Enzymol. 304, 3−19.

27. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44−57.

28. Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFID to nucleosomes by chromatin association.

29. Wilm, M. (2009) Quantitative proteomics in biological research. Proteomics 9, 4590−4605.

30. Liu, H., Lin, D., and Yates, J. R., 3rd (2002) Multidimensional separations for protein/peptide analysis in the post-genomic era. BioTechniques 32, 898, 900, 902 passim.

31. Wysocka, J., Swigut, T., Milne, T. A., Dou, Y., Zhang, X., Burlingame, A. L., Shi, X., Hong, T., Walter, K. L., Ewalt, M., Michishita, E., Hung, T., Carney, J. F., Mi, L. Z., Chruszcz, M., Cymborowski, M., Clines, K. L., Feng, W., Yonezawa, M., Ye, J., Jenuwein, T., and Grummt, I. (2010) PHF8 interacts with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development.

32. Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFIID to nucleosomes by chromatin association.

33. Allis, C. D., Jenuwein, T., Reinberg, D. (eds.), Caparros, M. L. (assoc. ed.) (2006) Epigenetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

34. Wilm, M. (2009) Quantitative proteomics in biological research. Proteomics 9, 4590−4605.

35. Karagianni, P., Amazit, L., Qin, J., and Wong, J. (2008) ICBP90, a novel methyl K9 histone binding protein ubiquitination with hetero-chromatin formation. Mol. Cell. Biol. 28, 705−717.

36. Fischle, W., Franz, H., Jacobs, S. A., Allis, C. D., and Khorasanizadeh, S. (2008) Specificity of the chromodomain Y chromosome family of chromo-domains for lysine-methylated ARK(S/T) motifs. J. Biol. Chem. 283, 19626−19635.

37. Vorbruggen, M., and Galperin, M. Y. (2010) The 2010 Nucleic Acids Society meeting: resources for bioinformatics. Nucleic Acids Res. 38, D1−D22.

38. Vyas, S. K., and Hennig, S. (2009) The HP1 chromo shadow domain binds a consensus peptide. PLoS ONE 4, e5786.

39. de Jager, J., and Kingston, R. E. (2009) Chromatin higher-order structure and protein interactions. Nat. Struct. Mol. Biol. 16, 1181−1185.

40. Nagler, A., von Eggelkraut-Gottanka, R., Klose, A., Beck-Sickinger, A. G., and Beyer-Grummt, I. (2010) PHF8 interacts with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development.

41. D'Andrea, A. D. (2010) Susceptibility pathways in Fanconi's anemia and breast cancer. N. Engl. J. Med. 362, 1909−1919.

42. Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFID to nucleosomes by chromatin association.

43. Enan, D. C., Jenuwein, T., Reinehr, D. (eds.), Caparros, M. L. (assoc. ed.) (2006) Epigenetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

44. Wilm, M. (2009) Quantitative proteomics in biological research. Proteomics 9, 4590−4605.

45. Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFID to nucleosomes by chromatin association.

46. Wilm, M. (2009) Quantitative proteomics in biological research. Proteomics 9, 4590−4605.

47. Vyas, S. K., and Hennig, S. (2009) The HP1 chromo shadow domain binds a consensus peptide. PLoS ONE 4, e5786.

48. de Jager, J., and Kingston, R. E. (2009) Chromatin higher-order structure and protein interactions. Nat. Struct. Mol. Biol. 16, 1181−1185.

49. Nagler, A., von Eggelkraut-Gottanka, R., Klose, A., Beck-Sickinger, A. G., and Beyer-Grummt, I. (2010) PHF8 interacts with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development.

50. Nagler, A., von Eggelkraut-Gottanka, R., Klose, A., Beck-Sickinger, A. G., and Beyer-Grummt, I. (2010) PHF8 interacts with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development.
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