Biochimica et Biophysica Acta 1839 (2014) 657–668

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Biochemical systems approaches for the analysis of histone modification readout☆

Monica Soldi, Michael Bremang, Tiziana Bonaldi *

Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, 20139 Milan, Italy

ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form 6 March 2014
Accepted 18 March 2014
Available online 27 March 2014

Keywords:
Mass spectrometry
Proteomics
Histone post-translational modification
Chromatin
Histone code readers
Epigenetics

ABSTRACT

Chromatin is the macromolecular nucleoprotein complex that governs the organization of genetic material in the nucleus of eukaryotic cells. In chromatin, DNA is packed with histone proteins into nucleosomes. Core histones are prototypes of hyper-modified proteins, being decorated by a large number of site-specific reversible and irreversible post-translational modifications (PTMs), which contribute to the maintenance and modulation of chromatin plasticity, gene activation, and a variety of other biological processes and disease states. The observations of the variety, frequency and co-occurrence of histone modifications in distinct patterns at specific genomic loci have led to the idea that hPTMs can create a molecular barcode, read by effector proteins that translate it into a specific transcriptional state, or process, on the underlying DNA. However, despite the fact that this histone-code hypothesis was proposed more than 10 years ago, the molecular details of its working mechanisms are only partially characterized. In particular, two questions deserve specific investigation: how the different modifications associate and synergize into patterns and how these PTM configurations are read and translated by multi-protein complexes into a specific functional outcome on the genome. Mass spectrometry (MS) has emerged as a versatile tool to investigate chromatin biology, useful for both identifying and validating hPTMs, and to dissect the molecular determinants of histone modification readout systems. We review here the MS techniques and the proteomics methods that have been developed to address these fundamental questions in epigenetics research, emphasizing approaches based on the proteomic dissection of distinct native chromatin regions, with a critical evaluation of their present challenges and future potential. This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

1. Introduction

In the eukaryotic nucleus, the DNA strand exists in a highly compacted form achieved through winding around histone proteins, thereby forming a highly structured macromolecular complex, known as chromatin. The basic unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around an octamer made of two copies of each canonical histone protein (H3, H2A, H2B and H4) [1,2]. Histones are highly conserved from yeast to mammals, not only in their primary sequence, but also in their modification status. Histones are prototypes of hyper-modified proteins, and are subject to many distinct types of post-translational modifications (PTMs) [3], accumulating mainly at the unstructured *N*-terminus, which protrudes from the nucleosome [4].

The co-occurrence of various PTMs decorating core histones at specific genomic loci led to the proposal of a "histone code" [5]. According to this hypothesis, modifications on histones act sequentially or in combination to control the functional state of the underlying DNA [6–9]. In this sense, chromatin serves as a way not only to pack DNA but also to control when that DNA is used, through the regulation of its compaction and accessibility to factors that mediate various DNA-based processes.

To decipher the mechanism of action of the hPTM language, it is essential to understand how a single modification, or a combination thereof, is translated into a specific functional state. The biological functions associated with hPTMs are often exerted indirectly (*trans*-) through the recruitment or repulsion of specific proteins or multi-protein complexes that mediate the signal transduction of these marks through the execution of various DNA-based processes, such as gene expression, DNA replication or repair and cell cycle regulation [10–16]. In a few cases, however, this mechanism is direct (*cis*-), as it is for histone hyperacetylation that affects higher-order chromatin folding and hence its accessibility to nuclear factors both in vitro [17] and in vivo [18].

Since the first proposition of the histone code model, various methods have been designed to both dissect all existing hPTMs and

☆ This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

* Corresponding author at: Dept. Experimental Oncology, IEO, Via Adamello 16, 20136 Milan, Italy. Tel.: +39 0294375123.
E-mail address: tiziana.bonaldi@ieo.eu (T. Bonaldi).

http://dx.doi.org/10.1016/j.bbagrm.2014.03.008
1874-9399

Table 1
Strategies for isolation of specific chromatin regions/loci for subsequent proteomics characterization.

"Swapped-ChIP" approaches	Bait	Organism	References
PICh (Proteomics of Isolated Chromatin)	DNA probe complementary to telomeric-repeats	*H. sapiens* *D. melanogaster*	[87,88]
Minichromosome affinity purification	Immobilized-Lac repressor	*S. cerevisiae*	[89]
ChAP–MS (chromatin affinity purification with mass spectrometry)	LexA–PrA or TAL–PrA	*S. cerevisiae*	[90,91]
"Conventional ChIP" approaches	**Bait**	**Organism**	**References**
mChIP (modified chromatin immunopurification)	Tagged-histone variant or DNA-binding protein or chromatin-binding protein	*S. cerevisiae*	[98,99]
ChroP (chromatin proteomics)	hPTMs (through antibody)	*H. sapiens*	[100]
ChIP-MS (chromatin interacting protein-mass spectrometry)	Biotinylated-protein	*D. melanogaster*	[101]

identify the factors that recognize and bind them — the so-called "readers". Mass spectrometry (MS) and MS-based proteomics, especially in its quantitative format, have emerged as powerful approaches to gain a system-level view of the histone code. At the level of individual histones, MS can provide an unbiased and comprehensive map of protein modifications, allowing the detection of new modifications, revealing interplays among them, and providing an accurate measure of their quantitative changes in transitions between distinct functional states [19–23]. Many proteomics strategies have also been developed to characterize the composite architecture of chromatin [24–27], demonstrating that MS is a fundamental technological platform for research in epigenetics.

A deep comprehension of the histone modification functional readout requires an understanding of the distinct proteins that interact with specific hPTM patterns. These proteins act in concert with other players, such as other proteins, RNAs and methylated DNA, to enforce a defined configuration on chromatin.

Comprehensive approaches are needed to capture the complexity of the numerous physical and functional interactions. Among the various strategies available, those that combine chromatin immunoprecipitation (ChIP) with mass spectrometry seem particularly well-suited for this purpose, since they facilitate the parallel analysis of histone marks and their binding proteins at functionally distinct chromatin regions.

In this review, we will outline the major achievements of MS for the investigation of the histone code. We will then describe different functional proteomics strategies available for screening the histone readers. When finally focusing on the ChroP approach developed in our group, we will overview briefly the technical requirements to set-up the method and outline its strengths and limitations. Lastly, we will provide perspectives on developments required to provide the community with a system-level comprehension of the complex interplays between hPTM patterns, their readers and writers, chromatin architecture and the functional readout.

2. Overview of MS-proteomics approaches to read the histone code

2.1. Mass spectrometry for the annotation of the hPTM code

2.1.1. MS for PTM identification

Histone modifications have been traditionally studied by means of specifically-raised antibodies [28]. Although advantageous for their sensitivity, antibody-based assays require an a priori knowledge of the modification of interest. In addition, antibodies can suffer from cross-reactivity with similar modifications embedded within an identical amino acid context (e.g. K9 and K27 of histone H3) [29,30]. Moreover, epitope occlusion effects occur when the detection of a modification is inhibited by the presence of a mark on a neighboring site, as in the case of H3K9me/S10pho [31]. These events can be frequent in histones, due to their heavily modified nature.

In this respect, mass spectrometry (MS) can be very useful. In fact in MS a post-translational modification is detected essentially as a "delta-mass" (Δm) between the theoretical and experimentally-measured masses of a peptide. As such, any possible modification can be identified based on the accurate measurement of the Δm value, with virtually no limitations concerning the position, number and combinations of marks present on a polypeptide. Thus, MS distinguishes unambiguously between near-isobaric modifications and detects their combinatorial patterns, even on very long polypeptides and up to intact histones [32,33,19,34,35,21,36,37]. In addition, the detection of Δm implies that the technique is an unbiased and efficient means to discover novel marks. The last ten years have witnessed the publication of an increasing number of papers describing the successful use of MS for the annotation of up to 200 distinct modification types [38]. In those that are already known, new sites have been annotated [39,40], such as for: lysine acetylation, ubiquitylation and SUMOylation; different degrees of lysine and arginine methylation; serine, tyrosine and threonine phosphorylation; arginine citrullination; ADP ribosylation and carbonylation. In addition, MS-based approached have helped to identify novel modifications such as lysine propionylation [41] and crotonylation [40]; serine or threonine O-GlcNAcylation (beta-N-acetylglucosamine) [42–44]. The observation that some of these new marks have been discovered very recently suggests that the code is far from being exhaustively uncovered.

2.1.2. MS for quantitation of hPTMs

MS also functions efficiently in profiling hPTMs across multiple functional states, using different strategies: chemical labeling, in vivo metabolic labeling with isotope-encoded amino acids (SILAC) and SILAC-derived strategies, recently reviewed in [20,23,22,45]. SILAC was used to profile hPTM dynamics during the cell cycle [46], compare the marks decorating newly-synthesized versus "old" histones during protein synthesis [47] and characterize the time-dependent establishment of hPTMs after histone deposition on chromatin [48]. An adaptation of SILAC using a "heavy-labeled spike-in" approach was designed for the comparative analysis of hPTMs across several breast tumor samples [49]. Finally, heavy methyl SILAC (hmSILAC, [50]) was successfully employed to study histone methylation turnover [51–53]. A combination of both standard- and heavy methyl-SILAC was used to profile the dynamics of H3K79 methylation in the cell cycle [54] and the rates of transition between the different H3K27 and K36 methylation states [55].

2.1.3. MS to assess hPTM combinatorics

Mass spectrometry is particularly well suited to investigate the combinatorial association of different marks within the same molecules or even across different histones along nucleosome stretches. In a few specific cases, the co-existence or exclusion of distinct marks can be addressed by shotgun proteomics, upon the use of proteases with different specificities than trypsin. The endoproteinase Arg-C for example cleaves at the amide bond C-terminal to arginine residues and generates peptides containing multiple modified residues, such as the peptide aa (9–17) of histone H3 that allows the simultaneous detection of modifications on K9 and K14. Similarly, K27 and K36 methylations lie on the peptide aa (27–40) of H3, as well as the four acetylations on

the H4 *N*-terminal tail aa (4–17). However, connectivity over longer distances between *cis*- and *trans*- hPTMs remains elusive through bottom-up approaches because tryptic or Arg-C-derived peptides are typically too short. For example, although methylated K4 and K36 are both marks of active chromatin, it is not possible to gain hints on their coexistence on the same H3 molecules because of their distance along the *N*-terminus. The synergies between marks in the histone tails and those laying the globular domains are even less accessible to investigation by conventional bottom-up MS. In order to address this aspect, Jaffe and co-workers have described an interesting solution to assess hPTM combinations by bottom up MS: they carried out an immunoprecipitation of HPLC-purified soluble H3, using antibodies against distinct modifications (e.g. H3K9me3 and H3K4me3) and then analyzed PTMs by conventional MS analysis upon Arg-C-like digestion of the immunopurified H3. Upon proving the specificity and selectivity of the antibody, this method allowed the authors to infer the co-association of marks within the same molecule, even using a peptide-centric approach [56]. However, to date, this approach has not been followed by applications, mainly because of the success of middle- and top-down MS-based methods. These methods have been used to investigate the combinatorial aspect of the code through the analysis of large peptide fragments [57,58], up to intact histone molecules [59–61]. Kelleher and collaborators have introduced tandem mass spectrometry directly on intact histones, by means of top-down analysis. Top-down MS relies on a variety of fragmentation techniques, which largely depend on the intrinsic properties of the protein ions, such as size, charge and conformation in gas phase. Electron capture (ECD) or Electron transfer (ETD) dissociations are the most frequently adopted techniques for the fragmentation of intact proteins because they are particularly efficient with high charge state ($z > 3$) peptides. Through a number of studies, top-down MS has achieved information about all hPTM combinations and stoichiometries on the four core histones [62–67]. However, a major limitation of ECD is the high number of ions generated in a single fragmentation event, which requires several tandem-spectra to reach a reasonable signal-to-noise ratio, thus reducing the sensitivity and compatibility with the liquid chromatography time-scale. Middle-down approach has emerged as a compromise between bottom-up and top-down approaches. It enables the analysis of longer peptides (e.g. the complete *N*-terminal tail of both H3 and H4) by using proteases with few digestion sites on histones. Middle-down has been used to successfully characterize the modification patterns and relative stoichiometries of histones H3 and H4 in HeLa cells [57,58,34], and, more recently, in mouse embryonic stem (ES) cells [68]. Overall, however, both top-down and middle-down approaches present the challenge of deconvoluting highly complex tandem mass spectra. Specialized software is required to assign multiple co-occurring PTMs to specific sites with high confidence and to summarize their complex combinatorial patterns [69–71]. This issue has limited their dissemination beyond the circle of groups with expertise in this branch of MS and interests in epigenetics.

An interesting future perspective might be provided by the recent application of the Orbitrap mass analyzer within the Exactive instrument to measure protein assemblies up to one megadalton in size [72]. This implementation opens up the possibility of carrying out top-down proteomics on the intact histone core octamer, with the possibility of inferring both inter- and intra-molecular PTM associations among the different core histones. In fact this platform was also shown suitable for resolving and quantifies complex glycosylation patterns.

The overall limitation of MS-based analysis of histones is the use of bulk chromatin preparations as starting sample due to the substantial amount of material required for the MS-detection of sub-stoichiometric modifications. Consequently, the studies described have provided insights only into global modification changes associated with cellular functional transitions, like developmental switches, cell-cycle stages, depletion of gene products, and chemical inhibitions of specific histone-modifying enzymes, but they have fallen short in assessing distinct hPTM patterns at functionally distinct genomic loci. In this sense, analytical solutions to carry out hPTM analyses of physically more restricted chromatin regions are highly desirable.

2.2. Screening of hPTM readers

2.2.1. Identification of readers by affinity pull-down SILAC screening

MS-based interactomics has been successfully employed in a number of biochemical assays aimed at screening for histone code readers. These types of experiments permit an unbiased, proteome-wide screening of binders of distinct modifications on histones, providing a useful alternative to educated approaches in which readers are extrapolated on the basis of protein domains known to mediate specific hPTM–protein interactions [73–76].

The general experimental design, common to all different proteomic-affinity interaction strategies, can be synthesized as follows: an interaction profiling is carried out based on a pull-down experiment where nucleosolic extracts are incubated with selected components of chromatin — bearing single modifications or combinations thereof. This is followed by LC-MS/MS-based identification of the proteins retained on the solid support (typically beads), which are classified as potential binders when they are measured as enriched relative to the unmodified version of the same bait (the mock control). Since one feature of protein–protein interactions mediated by PTMs is the relatively low affinity, the identification of hPTM readers by conventional proteomics may be challenged by the difficulty in enriching and discriminating specific interactors from co-purified "hitch-hiker" proteins. In this sense, coupling affinity-based enrichment with quantitative proteomics methods, such as SILAC, has significantly enhanced the efficiency of these screenings.

Allis and co-workers first conceived the scheme of this assay, however using conventional single gel-band MS-analysis to identify WDR5 and BPTF, which appeared as enriched protein bands in a Coomassie SDS-PAGE, upon a pull-down using a histone H3 peptide bearing trimethylation on lysine 4 (H3K4me3) [77,78]. Through a collaborative effort, Vermeulen et al. used the same affinity pull-down assay using peptides mimicking modified H3-tails, but combined it with SILAC to annotate the complete repertoire of H3K4me3-interacting proteins by shotgun proteomics [79] (Fig. 1A, left). This study not only confirmed previous results but also identified novel readers of H3K4me3. For instance, the entire TFIID complex is recruited at this modification through the binding of TAF3 via its plant homeodomain (PHD) finger. This approach was then extended by carrying out a comparative study on a panel of peptides bearing tri-methylation at distinct lysines of H3 (K4, K9, K27 and K36) and of H4 (K20), leading to the acquisition of the histone lysine tri-methyl-interactome [80]. The combination of SILAC-interactomics with ChIP-Seq profiling and BAC-GFP transgeneOmics helped to reconstruct the complex architecture of these interactions and to gain mechanistic insights into the regulation of gene expression mediated by these modifications.

Along the same lines, Kapoor and co-workers developed the CLASPI approach, where the SILAC pull-down is implemented through the use of photo-cross-linking peptide probes that mimic methylated histone tails. This approach has the advantage of converting weak protein-PTM interactions into covalent ones, which allows one to capture also additional binders that had escaped non-covalent screenings [81].

Following these initial studies, two main implementations of the pull-down assay have been characterized by the use of more sophisticated baits, with the rationale that some interactions between histone marks and their respective readers may be influenced by the native environment. Low affinity readers could indeed bind more specifically and efficiently to hPTMs embedded within the whole histone molecule, up to the intact nucleosomes, compared to modifications present on linear peptides. In fact, it has already been observed that certain histone modifying enzymes (HME) do not recognize peptides as substrates

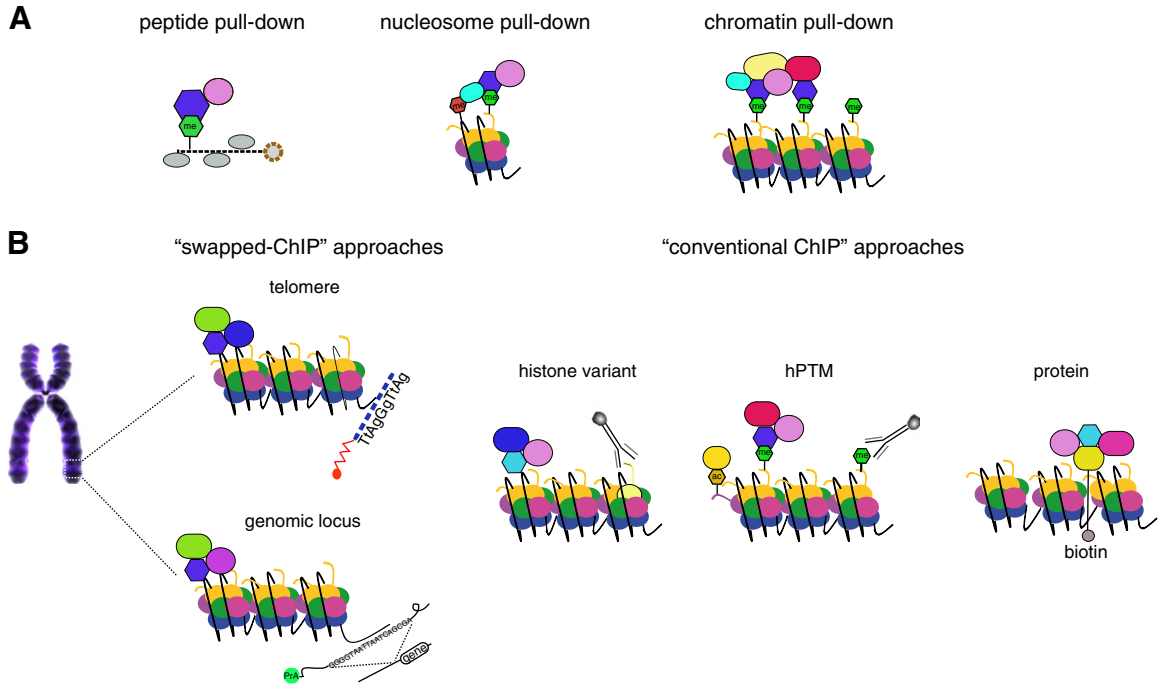

Fig. 1. Strategies for enriching hPTM- and chromatin-binding proteins. A) Pull-down based approaches to characterize readers of hPTMs using different baits: immobilized histone modification peptides (left), recombinant nucleosomes bearing a combination of hPTMs and methylated DNA (middle) or recombinant modified chromatin (right). B) "Swapped-ChIP" strategies allow isolating specific DNA domain or native locus, using either a complementary DNA probe (upper) or TAL effector protein matching to a specific chromosome section (i.e. the promoter region of GAL1) (lower), respectively. Conventional chromatin immunoprecipitation strategies enable to isolate chromatin sections using antibodies against histone variants (left), hPTMs (middle), or biotinylated proteins (right) as bait. Green and orange hexagons correspond to methylated and acetylated histone protein, respectively. Red hexagon corresponds, instead, to methylated DNA.

while they do so on intact histone or nucleosomes [82]. Recombinant mono-nucleosome templates, reconstituted in vitro starting from DNA stretches and recombinant histones, uniformly modified at specific residues, have been employed to assess the combined contribution of both DNA and histone methylations in recruiting specific readers onto chromatin (Fig. 1A, middle). This approach, named SNAP (SILAC nucleosome affinity purification), revealed that UHRF1 and ORC bind the H3K9me3-modified nucleosomes when the CpG methylation is present. In contrast, the H3K36 demethylase KDM2A is enriched in H3K9me3-nucleosomes but its recruitment is inhibited by DNA methylation, thus highlighting the real combinatorial effect of DNA and PTM modifications in recruiting proteins [83]. Similarly, nucleosome arrays, where a recombinant chromatin template uniformly tri-methylated on H3K4 and H3K9, or mono-ubiquitylated on H2BK120, were used in SILAC-based affinity purification experiments (Fig. 1A, right) [84,85]. The observation of a limited overlap between the interactomes obtained using as baits either chromatin or histone peptides bearing the same modification corroborates the initial intuition that the context in which an hPTM is embedded can have a significant impact on the recruitment of certain readers and suggests that in vitro approaches to investigate the histone readout may have intrinsic biases.

With respect to educated approaches, affinity-interactomics strategies are comprehensive, having the advantage of unraveling not only direct but also secondary interactions, as for the recruitment of multi-protein complexes, illustrated in the case of TFIID bound to H3K4me3 peptides [79]. Furthermore, the use of recombinant, in vitro re-constituted systems offers the opportunity of manipulating precisely the type, composition and combination of modifications, which, in turn, enables the validation of experimentally well-defined synergisms or antagonisms among epigenetic marks. For instance, the synergy of H3K4me3 with H3K9/K14 acetylation in recruiting TFIID and BPTF has been demonstrated, while H3S10 phosphorylation was shown to destabilize CDYL but not HP1 binding to H3K9me3 [80]. On the other hand, major limitations reside in the fairly artificial conditions of the screening. For example, the modifications of these recombinant templates are stoichiometric and thus these methods do not reproduce their physiological abundances, which instead are in several cases sub-stoichiometric compared to the unmodified counterpart, as observed in a number of MS-studies where the relative abundance of PTMs was estimated through intensity-based quantitation of unmodified and modified peaks [49,86,65]. This can impact the actual binding affinity of the readers. Hence, although useful and informative in the discovery phase, up to now these in vitro approaches have not been exploited to their maximum potential, by taking into account both the real modification abundance and arrangements and all other chromatin determinants existing at specific regions, to generate a picture that would reflect more faithfully the native code-to-reader interplays.

2.2.2. Methods based on the isolation of native chromatin

With respect to in vitro affinity screening, approaches based on the proteomics dissection of affinity-enriched native chromatin have the advantage of enabling the parallel investigation of both hPTMs and readers natively co-occurring at defined regions (See Table 1). The first example of a strategy based on biochemical isolation of a physically and functionally discrete chromatin region for the subsequent characterization of its protein composition by MS is the PICh (Proteomics of Isolated Chromatin) approach — an elegant method where the purification of cross-linked human and *Drosophila melanogaster* telomeres is achieved using DNA probes complementary to these regions [87,88]. One limitation of PICh is that its application is restricted to regions rich in repetitive DNA sequences. However, the PICh approach is a milestone because it demonstrated for the first time that chromatin affinity-purification can be successfully combined with MS-proteomics, and thus paved the way for the design of other strategies based on the same "swapped-ChIP" rationale. In contrast to conventional ChIP, where proteins or PTMs are used as baits to isolate DNA before qPCR profiling or sequencing, the logic of "swapped-ChIP" is to use DNA as the baits, or "handles", for sequence-specific isolation of distinct chromatin regions, specific to a

genomic region (Fig. 1B). The composition of isolated proteins is then dissected by high-resolution mass spectrometry.

A number of assays have been established in *Saccharomyces cerevisiae*, a model system that can be genetically engineered to generate solutions for isolation of chromatin pieces. Tsukiyama and co-workers generated from the yeast genome a TRP1–ARS1–LacO mini-chromosome that contains the TRP1 gene, an efficient early-firing origin of replication gene and the lac operator sequence (LacO) [89]. Using an immobilized lac repressor, they affinity-purified the mini-chromosome from whole cell extracts for subsequent MS-analysis of both the hPTMs and proteins associated with the origin of replication at specific stages of the cell cycle. This approach uncovered that the dynamic regulation of histones H3 and H4 amino-terminal acetylation around ARS1 facilitates firing of endogenous origins at chromosomal locations, thus correlating a set of hPTMs with DNA replication.

With a similar strategy, the group of Tackett presented ChAP–MS as the approach that truly combines ChIP and MS to investigate both proteins and histone post-translational modifications associated to a single genomic locus [90]. The GAL1 locus from budding yeast in transcriptionally silent and active conditions was purified through the use of a single genomic LeX-A DNA binding site. The DNA affinity handle was inserted just upstream of the GAL1 start codon via homologous recombination in a strain constitutively expressing a LexA–ProteinA (LexA–PrA) fusion protein. Thus, the strain contains an ectopically expressed affinity-tagged LexA protein with an incorporated DNA affinity handle for site-specific chromatin isolation. The yeast strain was cultured in either glucose or galactose to achieve repression or activation of the GAL1 gene, respectively. In addition, the authors combined the LexA–PrA purification with quantitative proteomics based on iDIRT (isotopic differentiation of interactions as random or targeted) labeling to discern specific interactors from background proteins. However, the authors did not carry out the MS-based analysis of PTMs of histones from the enriched chromatin, hence the comprehensive view of locus-specific hPTM patterns remained unaddressed and it represents an attractive aspect to investigate in the future. A limit of ChAP–MS is that it operationally relies on genetic engineering of the budding yeast genome, possible in this model system but less straightforward in higher eukaryotes. However, the method is conceptually very attractive and suitable for interesting applications. One of them is the TAL–ChAP–MS approach, an implementation that allows enrichment of native genomic loci without genetic engineering [91]. Taking advantage of the transcription activator-like (TAL) ProteinA as an affinity handle, a small section of chromatin upstream of the GAL1 locus was isolated and subsequently MS-analyzed to identify histone marks and proteins mediating transcriptional activation of the gene. Label-free quantitative proteomics was used instead of an isotope-labeling approach for protein quantitation. The possibility to 're-program' the DNA-binding specificity of the TAL protein to obtain a unique affinity purification reagent for each chromosome region of interest holds great promises in enabling the widespread investigation of chromatin composition and epigenetic states of various yeast genetic loci. However, its application for in-depth analyses of hPTMs and to other model organisms has not yet been described.

Alternatives to the "swapped-ChIP" strategy include methods that use conventional chromatin immunoprecipitation (ChIP) (Fig. 1B) to isolate distinct genomic regions for subsequent proteomic analysis. In its usual set-up, and through the analysis of the isolated DNA using PCR, DNA microarray (ChIP-on-chip) or deep sequencing (ChIP-Seq), ChIP allows the acquisition of genome-wide profiles of various chromatin-components with a resolution of a few nucleosomes [92–97]. The strengths of ChIP-Seq are: the dissemination of the method among many laboratories; its robustness, achieved through several years of optimization of reagents (antibodies), protocols (cross-linking, immuno-affinity step), data analysis and the creation of data repositories for the sharing and comparison of results. The major drawback is that individual marks are analyzed in each experiment, which limits the assessment of multiple PTM types and the co-occupancy of interactors on single nucleosomes.

In the proteomics equivalent of ChIP, the immunoprecipitation is followed by MS-analysis to identify qualitatively and quantitatively the hPTMs, histone variants and chromatin-binding proteins that are isolated together with the modification or protein used as "bait". ChIP-MS methods have the advantage of being directly derived from standardized protocols of cross-linked chromatin immunoprecipitation, which should facilitate their adoption among scientists. However, the adaptation of conventional ChIP to proteomics has remained at a concept stage for long and only recently been achieved, upon a few technological achievements. First, it has become possible to analyze proteins obtained from formaldehyde cross-linked samples. Second, the higher sensitivity and increased dynamic range of recent mass spectrometers have facilitated detection of low abundance, sub-stoichiometric post-translational modifications from small quantities of starting histones. In this respect, it is worth mentioning that, despite these improvements in instrumentation and the considerable scale-down of ChIP experiments, the starting material required for in-depth proteomics analyses of affinity-isolated chromatin is still 1–2 orders of magnitude more than what is required for traditional ChIP-Seq.

The first method that merged ChIP with MS was described by Figey and co-workers in 2007, and named mChIP (modified chromatin immunopurification). This approach consists in the isolation of chromatin by affinity-enrichment of DNA-bound proteins; it was applied to the analysis of the interactome of nucleosomes containing the *S. cerevisiae* histone variants Hta2p and Htz1p [98]. The same authors followed up the technique with the first large-scale mChIP characterization of the chromatin interactome in budding yeast using 100 DNA- or chromatin-binders as baits. MS was used to identify the novel chromatin proteins associated with these baits, generating of a vast network of novel associations grounded on chromatin [99]. The limitations of the mChIP approach are twofold: first, it lacks quantitative methods to discern specific interactors from the classical chromatin background. Second, the histone code readout was not dissected, because the study was not focused on the analysis of hPTM patterns on the enriched chromatin.

Recently, our group has implemented the ChroP approach where a standard ChIP, using as baits two marks associated with functionally distinct chromatin regions (H3K9me3 and H3K4me3), was combined with MS-SILAC based quantitative proteomics. This approach allowed the comprehensive investigation of the histone modification and variants as well as the co-interacting chromatin proteins that co-associate at specific regions to enforce definite functional states (the so-called "chromatome") [100]. Being based on the enrichment of intact mononucleosomes at quasi-purity, ChroP enables observations of intermolecular synergisms between modifications decorating different core histones within the same nucleosome. In addition, it allows for the first time assessments of the region-specific compartmentalization of histone variants and linker histone subtypes. Since the investigation of such proteins has been held back by the lack of good quality antibodies, ChroP is uniquely efficient in investigating their genomic distribution and specific role. The ChroP protocol is essentially the same used in ChIP-Seq, thus the two assays are highly complementary. We envisage that a single scale-up ChIP experiment can be carried out and a minor fraction of the sample is used for deep sequencing, while the remaining is subjected to MS analysis. ChroP and ChIP-Seq are two sides of the same coin: the former dissects the physical associations among distinct determinants of chromatin and the latter indicates the genome-wide localization of such composite architectures.

However, the current version of ChroP suffers from some restraints, outlined in Section 4, which will be overcome through future technical and functional implementations of the method (see 4.1 and 4.2). For instance, the current examples of ChroP's application in the literature imply the use of trypsin and Arg-C to digest enriched histones prior to MS. In this way, long-distance synergies have to be inferred from the data, rather than being documented by direct observed co-occurrence

of PTMs within one polypeptide (e.g. co-occurrence of PTMs at K4/K9/K29/K36). Thus, distinguishing *cis*- and *trans*-occurrences and inter/intra-co-occurrence of PTMs on histone molecules is not possible, in the present setup. In perspective, the combination of ChroP with the use of top-down and middle-down MS strategies will enable one to assess long-distance PTM synergisms (see 4.2). In addition, the use of ChIP-grade antibodies against hPTMs in ChroP is at the same time advantageous, as it makes the method an exact complement to ChIP-Seq, and limiting, since most antibodies are polyclonal thus heterogeneous in their efficiency/specificity, which could affect ChroP robustness. Consequently we have experienced that ChroP needs case-by-case optimization, depending on the antibody specificity and modification abundance in the cell type under investigation (see paragraph 3.2 for more details).

In this light, there is promise in those strategies that take advantage of in vivo tagging of endogenous proteins/modifications to generate a standardized affinity-purification method. The ChIP-MS (chromatin interacting protein-mass spectrometry) method, described by Kuroda and co-workers, uses as bait an in vivo biotinylated version of *D. melanogaster* MSL3 and MSL2 proteins, upon the insertion of a bacterial HBT tag into their genes [101]. Since MSL2 and 3 are subunits of the fruit fly Dosage Compensation Complex (DCC), the MS analysis of the isolated cross-linked chromatin leads to the proteomic dissection of MSL-bound chromatin. Through this strategy two novel candidate subunits of the complex were identified (CG1832 and CG4747 proteins) and validated by functional assays. The authors used a TAP-tagged version of MSL3 to carry out the analysis of MSL-associated hPTMs. They found that H4K16 acetylation is a hallmark of DCC-bound chromatin and that methylated H3K36 and H3K79 are also strongly enriched, in line with previous literature [102,103,92,104,105]. This work on DCC reveals potential novel determinants of the transcriptional state of the male X chromosome genes and offers insights onto novel hPTM-reader associations, such as the one between H3K36me3 and CG4747. However, possible limitations include the lack of a comprehensive quantitative analysis of hPTMs across the distinct core histones of the DCC-bound nucleosome, and the use of genetically engineered MSL2 and MSL3 genes for tag-based affinity chromatin purification. Even if this is straightforward in fruit fly, the strategy may be more laborious in mammals.

3. ChroP for region-specific dissection of chromatin proteomic composition

3.1. Essentials of N-ChroP and X-ChroP protocols and representative results

ChroP articulates into two distinct protocols, depending on the question addressed by MS and the corresponding type of chromatin used as input. In N-ChroP, unfixed, native chromatin digested with MNase is employed to purify mono-nucleosomes from bulk chromatin and to evaluate the co-enriched hPTM patterns. Instead, X-ChroP consists in the affinity-isolation of cross-linked sonicated chromatin fragments carried out in conjunction with a peptide competition assay and SILAC-based quantitative proteomics to discern genuine binders from unspecific background proteins [106–108,79]. Technical details of X-ChroP and N-ChroP protocols are provided [100,109]; hence, in this section we will offer a more conceptual overview of the crucial steps in the procedures, illustrating their similarities and differences and describing the visualization of typical results.

N- and X-ChroP are comparable in their basic experimental design (Fig. 2A), aside from the use of native versus formaldehyde cross-linked chromatin and of unlabeled versus SILAC-labeled samples, respectively. Briefly, a ChIP-grade antibody against a specific histone modification is incubated with the fragmented chromatin. Proteins associated to isolated chromatin regions are recovered and processed prior to mass spectrometry. This step consists in protein separation by SDS-PAGE; in-gel digestion of proteins using site-specific proteases; extraction and de-salting of peptides that are finally separated by reversed-phase liquid chromatography and analyzed by tandem mass spectrometry (RP-LC–MS/MS). While the processing of MS raw data from N- and X-ChroP for both hPTM and protein identification is based on the use of a specific search engine (Mascot [110] and Andromeda [111], respectively), the relative quantification of histone modifications and of putative interactors is carried out in different ways. In particular, in N-ChroP, all identified hPTMs are validated by manual inspection of the corresponding MS/MS spectra. Label free quantification is achieved first by calculating the relative abundance (RA) of each modification using the signal intensity of the unmodified and modified species for the corresponding peptide and, secondly, by estimating the relative enrichment (RE) of each modification in the immunoprecipitated octamer relative to input. Once the enrichment of the modification used as bait is verified, the co-association or depletion of all other modifications is assessed, both at the intra-molecular level and at the inter-molecular level on co-purified core histones within the isolated nucleosome. This creates the so-called “region-specific modificome”, where positive and negative cross-talks between hPTMs are annotated and visualized through a heatmap display (Fig. 2B). In X-ChroP assays instead, specific co-isolated proteins are identified through a combination of SILAC and competition assays using an excess of the soluble histone peptide, bearing the modification, which is used as bait. The soluble peptide added in excess in one of the two SILAC-ChIP channels saturates the binding capacity of the antibody and thus “competes out” the large proportion of nucleosomes containing the bait and, accordingly, all specific interactors that are discerned from background based on their specific SILAC H/L ratio (Fig. 2C).

3.2. Fine tuning of the procedure, with critical steps and tricks

The ChroP protocol was conceived directly from the standard chromatin immunoprecipitation protocol [112], with minor adaptations to MS. Yet, a number of critical aspects have been addressed during the set-up of the method; this section illustrates them, alongside with the solutions implemented and critical assessment on their benefits.

In ChroP, the length of the native nucleosome array obtained by either enzymatic (MNase) or mechanical (sonication) fragmentation of chromatin is very important and must be established depending on the different aims of the analysis. We chose to use as input for N-ChroP a fraction almost completely enriched in mono-nucleosomes, because we were particularly interested in dissecting the physical association of hPTMs across different core-histones within the distinct intact mono-nucleosomes. To ascertain that isolated nucleosomes are intact, the appropriate stoichiometry of the four different core-histones in the immunopurified material was carefully evaluated in the Coomassie-stained gel. A lack of the proper stoichiometry is indicative of partial disruption of the nucleosome, with impossibility of investigating inter-molecular cross-talks.

Comparing the PTM patterns obtained using nucleosome stretches of different lengths to be used as input for N-ChroP is an interesting development of the technique that enables assessment of the frequency of distinct PTM combinations along chromatin. For such analysis, homogeneous nucleosome stretches of precise length must be used, which can be purified through CsCl ultracentrifugation [113] or sucrose gradient centrifugation [114,115] of MNase-digested chromatin. Reinberg and co-workers have recently developed this idea, using a chromatin preparation containing 94% mono-nucleosomes, purified through sucrose gradient centrifugation, as input for the immunoprecipitation step, to assess if the two copies of histones are symmetrically or asymmetrically modified in vivo [116]. They could demonstrate that nucleosomes in ES, MEF and HeLa cells can exist in both symmetrically and asymmetrically modified forms for H3K27 di-/tri-methylation and H4K20 mono-methylation. Moreover, they propose that the activity of the Polycomb repressive complex-2 (PRC2) is inhibited only when the nucleosomes contain symmetrically active marks, such as

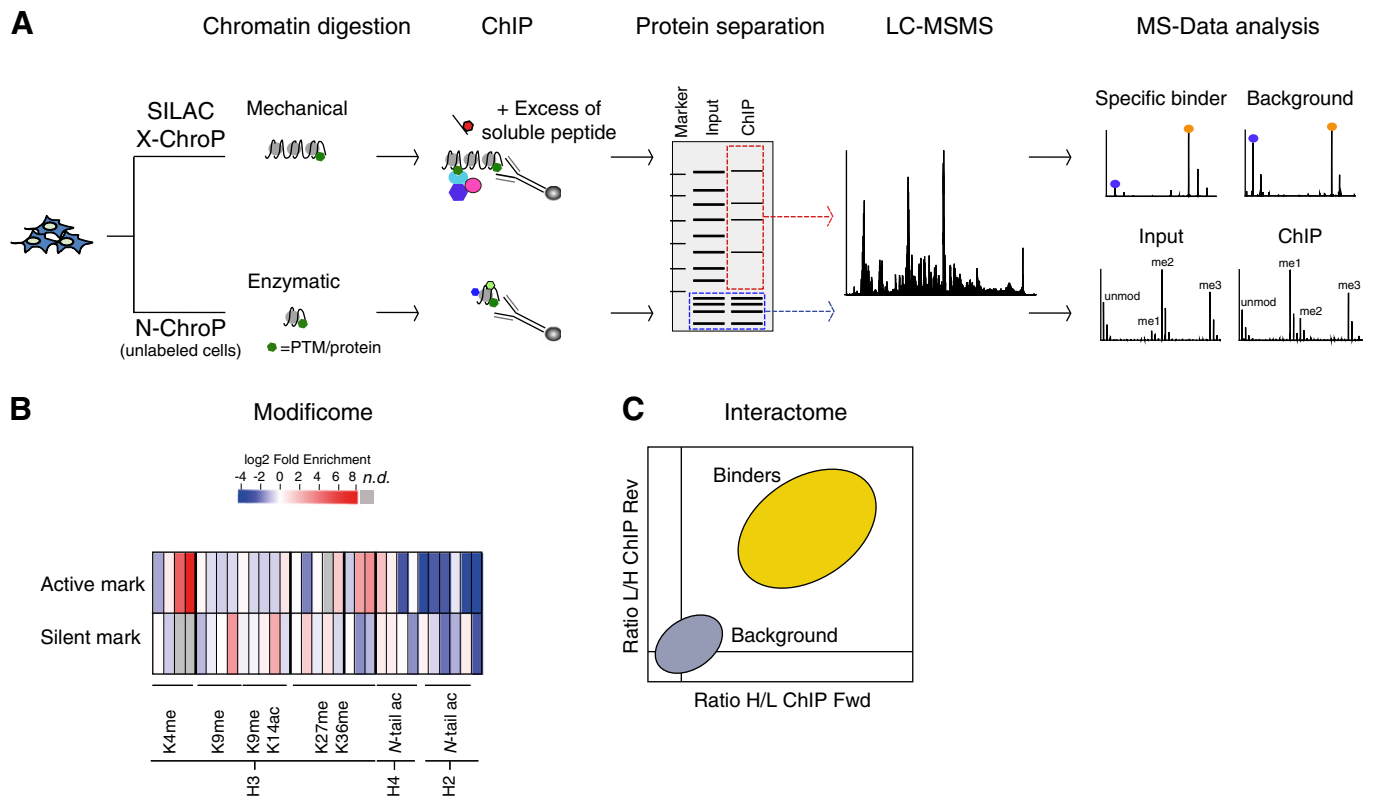

Fig. 2. ChroP approach for identifying and quantifying hPTMs or binding proteins co-associated with functionally distinct chromatin regions. A) Schematic overview of the ChroP workflow. Chromatin from labeled or unlabeled cells is fragmented using sonication (upper panel) or MNase (bottom panel). Purification of specific functional chromatin domains is obtained by incubating the chromatin with antibodies against either a specific hPTM or a non-histonic nuclear protein; in the X-ChroP approach one of the two chromatin samples is incubated with the same antibody saturated with an excess of soluble peptide. Immunopurified proteins are separated by SDS-PAGE and in-gel digested and analyzed by LC-MS/MS. Finally, MS raw data are analyzed, defining the SILAC ratio of the co-immunopurified proteins (upper panel) or comparing the relative abundance of hPTMs in immunoprecipitated material relative to input (bottom panel). B) The enrichment/depletion of co-associating hPTMs identified on histones H3, H4 and H2 is shown through a heatmap representation, where each row corresponds to a different modifications (n.d.: not detected modifications), while column corresponds to distinct chromatin regions. C) Proteins co-enriched within the same regions can be discerned from background based on their SILAC-ratio: background proteins (gray) cluster around protein ratio 1, while specific binders are in the top-right quadrant of the scatter plot (yellow).

H3K4me3 or H3K36me3. Thus, chromatin fragments of uniform length allow a more accurate quantification of the relative stoichiometry of modifications, both at the intra- and inter-molecular level, elucidating the real co-association or depletion between the different hPTMs. In X-ChroP we chose 300–500 bp chromatin stretches as input for ChIP, reasoning that multi-protein complexes recruited at modified chromatin typically include distinct proteins containing binding modules, which interact either with modifications present at distinct sites along the same histone molecule, or on different core histones, or even on molecules in neighboring nucleosomes. In any case, when nucleosomes of different lengths are under investigation, an increase or decrease in enzymatic or mechanical fragmentation is used to obtain chromatin enriched in mono- or poly-nucleosomes, respectively. While MNase digestion of native chromatin can be tuned more finely to achieve more homogeneous and reproducible nucleosome stretches, the precise control of formaldehyde-fixed chromatin sonication is challenging. Testing different cross-linking conditions or agents will in the future permit enzymatic digestions of fixed-chromatin, thus combining the advantages of cross-linking stabilization of low affinity and transient interactions (required when the bait is a DNA- or histone-binding protein) with the higher reproducibility of enzymatic digestion.

Another crucial step is the definition of the optimal ratio between the amounts of input chromatin and of antibody, which changes case by case, depending on the relative abundance of the hPTM/protein used as bait and on the antibody efficiency. Optimization is typically achieved experimentally, by comparing the abundance of the bait in input and its residual in the flow-through (FT) upon ChIP. If the bait is sufficiently depleted in FT (at least 50–60% depletion) one can trust that a significant proportion of the region of interest is enriched, thus excluding a bias due to the analysis of a not representative sub-fraction.

Finally, the optimal molar excess of the soluble peptide with respect to the antibody must be carefully titrated case by case. Also in this case the optimization is reached experimentally, by performing a competition ChIP assay with increasing concentrations of the excess peptide followed by an evaluation of the amount of bait in the immunoprecipitated samples and in the FT. We found that the ideal excess of soluble peptide typically reduces about 20 times the amount of bait retained on the beads. This number is, on the one hand, sufficiently large to benefit from the SILAC discriminating power, and on the other hand, not too extreme to induce the complete eviction of specific binders from the antibody, which would cause missing H/L ratios for quantification and statistical analysis.

4. Present limitations and future implementations of ChIP-based proteomics analyses of the histone PTM readout

Approaches based on chromatin-IP followed by the MS-investigation of region-specific chromatin determinants have so far been described in proof-of-principle studies, where the validation of already known markers/determinants of well-characterized chromatin regions has corroborated the robustness of the strategies. We envisage that a number of implementations will be beneficial to gain a more comprehensive

systems view of the histone modification readout. Refinement of the methods can affect both technical aspects and functional applications. We will offer some examples of possible developments, being aware that other ideas can stem from the creativity of individuals or the urge to answer specific biological questions.

4.1. Technical/biochemical implementations

A needed extension in order to make the ChroP a closer complement of ChIP-Seq for all protein determinants of chromatin, involves the use of either DNA-binding proteins or secondary chromatin interactors which recognize either hPTMs or DNA-bound factors, as baits. This implementation should be straightforward for all the factors for which a conventional ChIP has already been established, upon case-by-case optimization and tailoring the protocols for chromatin cross-linking to further stabilize secondary interactions and thus increase immunoprecipitation efficiency.

Applying ChroP/ChIP-MS strategies to in vivo tagging systems of endogenous proteins is highly appealing, due to the possibility of using a universal tag for immuno-affinity capture, which would facilitate the standardization of the protocol into a "universal chromatin proteomics" approach. An example of a tagging strategy is the BAC-recombineering method, where endogenous proteins were expressed as GFP fusions in HeLa S3 and used for pull-down in conjunction with quantitative proteomics for a standardized multiplexed analysis of the mitotic interactome [117,118]. Similarly, a biotin tag can be inserted upon in vivo protein biotinylation, introducing an exogenous biotin ligase gene (BirA/biotinylation tag system) [119–121]. Recently the BICON (Biotinylation-assisted Isolation of CO-modified Nucleosomes) method has been described, which uses exactly this strategy: the histone-modifying enzyme MSK1 was biotinylated and streptavidin was used to purify co-modified nucleosomes [122]. In this study the analysis for co-associating modifications was assessed only by western blot approach, thereby lacking the precision gained by MS. Alternatively, in vivo biotinylation can also be achieved using the endogenous biotin ligase enzyme to biotinylate the target gene, as in ChIP-MS strategy [101].

The use of universal tags paves the way to another crucial implementation: namely the elution of immuno-enriched chromatin from the beads by competing with excess of the soluble tag. This is the first step towards the transition from in-gel to gel-free methods for pre-MS sample preparation, which will eventually permit the acquisition of whole region-specific chromatomes in one single LC-MS/MS run. Thanks to the new Orbitrap-based mass spectrometers, such as the Q-Exactive, which have high MS/MS identification rates, combined with ultra high-pressure liquid chromatography (UHPLC) for peptide separation prior to ESI [123–125], it is now possible to comprehensively analyze sub-proteomes without the need of pre-MS protein separation and with relatively short LC gradients. The possibility of eluting isolated pieces of chromatin in solution without the interference of the immunoglobulin chains will make it possible to multiplex chromatomics analysis upon preparative ChIP.

Recently, a genome-engineering tool based on the RNA-guided Cas9 nuclease [126] from the clustered regularly interspaced short palindromic repeats (CRISPR) system was used successfully to induce precise cleavages at defined endogenous genomic loci also in human and mouse cells [127,128]. The CRISPR/Cas system facilitates genetic engineering in comparison to other genome-editing technologies, like zinc fingers (ZNs) and transcription activator like effectors (TALEs), enabling a spectrum of different modifications of the genome [129]. Furthermore, multiple guide sequences can be encoded into a single CRISPR array, thus leading the simultaneous editing of several sites within the mammalian genome, expanding the range of applicability of RNA-guided nuclease technology. In this respect, the CRISPR/Cas system may represent in the future another useful tool to isolate specific genomic regions for subsequent proteomics investigation.

4.2. Functional implementations

Time-dependent studies are a very attractive functional application of ChroP. ChIP is carried out serially, at different time-points of a biological process that encompasses dynamic changes in chromatin composition and state. For instance, it would be extremely interesting to investigate the dynamics of hPTMs, variants and interactors clustering at specific chromatin regions during the differentiation of embryonic stem cells, or upon a perturbation that induces global transcriptional activation, such as an inflammatory stimulus that elicits transcriptional activation of a large set of genes in immuno-competent cells [130–133]. In such studies, triple SILAC can be elegantly used in different experimental set-ups to profile chromatin composition at multiple time-points, as was elegantly demonstrated for the analysis of the phosphoproteome dynamics upon EGF stimulus [134]. Alternatively, recent achievements in label-free quantification (LFQ) can be beneficial for the quantitative profiling of proteins and modifications across multiple samples [135, 65,33]. While static ChIP-MS analysis provides fixed snap-shots on locus-specific chromatin composition, time-dependent studies will boost the understanding of chromatin plasticity during transitions between different gene expression states, offering novel insights into the timing and sequential translation of the code into the corresponding functional readouts.

In this perspective, we count on the fact that in the future it will be possible to combine ChroP with targeted quantitative proteomics approaches [136–144] as an alternative to shotgun analysis. The rationale is that after a discovery phase, where conventional ChroP/ChIP-MS serves to annotate the molecular signature of discrete genomic regions, targeted proteomics will help to monitor a well-defined set of novel interesting determinants in a multiplexed experimental design, without the need for SILAC labeling. Using prototypic peptides for each determinant, it will be possible to estimate their locus-specific enrichment or depletion in time-depended analysis upon various perturbations (e.g. differentiation stimuli, depletion of histone modifying enzymes, structural components). Another advantage in using targeted proteomics is the possibility to define the correct stoichiometry between the protein subunits of a specific complex associated with different chromatin regions. Analogous to the relationship between ChIP-Seq and ChIP-PCR, where the former provides a global picture of the distribution of a certain factor and the latter a view on enrichment at a specific gene, ChroP-targeted proteomics will extend the functionality of ChroP by enabling a very focused view of the changes in the composite architecture of chromatin.

Finally, an interesting development originates from the limitation of bottom up MS in assessing long-distance cross-talks among histone modifications, as a consequence of the fact that trypsin and Arg-C produce short peptides containing a limited number of co-occurring marks. An obvious follow-up is to analyze the nucleosomes isolated by preparative ChIP through a combination of bottom-up, middle- and top-down MS, to achieve a bird-eye view of the long-distance connectivity among histone marks at specific chromatin regions [145]. Although appealing, this application is not straightforward and requires a number of technical modifications to the protocol. First, so far, middle- and top-down methods have been employed only on bulk histone preparation due to the significantly larger amount of starting material required. It remains to be verified whether a reasonable scale-up of ChroP can meet with an equivalent increase in top-down sensitivity to make this experiment feasible [61]. In addition, since middle- and top-down approaches analyze either intact molecules or long (25–50aa) peptides produced by using proteases that are typically not active in gel (e.g. Asp-N and Glu-C), there is a need to set-up alternative elutions that solubilize chromatin and make them compatible with gel-free protein separation, such as the GELFrEE (gel-eluted liquid fraction entrapment electrophoresis) system

[146,147]. Hence, the combination of ChIP-MS approaches with middle- and top-down is highly challenging and yet worth the effort, since it appears as the sole strategy capable of revealing all hPTM cross-talks associated with functionally-distinct chromatin regions and thus relating such patterns of histone post-translational modification with functional states.

4.3. Implemented methods for bioinformatics analysis of the chromatome

Although mass spectrometry technology has accelerated research in chromatin biology during the past decade, significant computational challenges still remain before we can obtain a system view of hPTM readout. Specifically, new methods are required for the quantitative exploration and visualization of hPTM combinations.

A number of bioinformatics tools have been developed that can interpret spectra from modified peptide sequences produced by bottom-up approaches, including sequences derived from histones. However, obtaining information on the combinatorics of hPTMs would be helped by MS-based proteomics approaches that produce information on much longer peptides or intact proteins, such as middle-down and top-down approaches described previously in this review. Although data analysis has benefited from the introduction of instruments with high mass measurement accuracy, algorithms available to deconvolute the highly complex mass spectra produced by these approaches are still lacking [148]. These spectra are complicated by the larger analyte size, the greater numbers of fragment ions and the presence of different structural isomers of the same modified proteins. Currently, there are two popular tools available for the analysis of middle- and top-down data: ProSightPTM [149], the first software developed for analysis of intact proteins, searches the Uniprot database which contains known modifications and variations in protein sequences. An extended version of Mascot called BIG Mascot [150] has also been developed, which increases the precursor ion mass limit from 16 kDa to 110 kDa. In these middle-down and top-down approaches it is clear that, although these methods can provide quantitative information on the stoichiometries of whole or larger sets of hPTM combinations, gaining accurate information on the nature and localization of hPTMs is currently only available through bottom-up approaches. While middle- and top-down methods and instrumentation continues to develop, is therefore clear that algorithms are needed that combine robustly identified and localized hPTMs with the stoichiometric information gained from comprehensive approaches.

5. Conclusive remarks: novel frontiers for a systems view of the hPTM readout

One cannot disregard that the information obtained from genomics and proteomics data on chromatin are highly complementary. A further challenge therefore lies in the development of additional bioinformatics methods that integrate these data and offer new formats of visualization that, ideally, retain information on the chromatin organization. This information has been lacking in the one-dimensional chromosome walks of current genome browsers, commonly associated with genomics methods such as ChIP-Seq [151]. With respect to visualization, some developments have already taken place in this direction, such as the Hilbert space-filling curve representation used by Kharchenko et al. [152]. In their work, the authors elegantly map a set of nine predicted chromatin states, based on a set of selected hPTM combinations, to folded views of fly chromosomes, thereby revealing domains that are biologically significant but would not be easily discerned from a linear view, such as those corresponding to transcriptional elongation in heterochromatic regions. One could envisage the benefits of defining specific combinations of hPTMs, obtained by MS-based proteomics approaches, in a nucleosome- and locus-specific manner, and then mapping this information to a spatially-organized region of the genome.

As these approaches would also include information on the writers and readers of hPTMs, and conceivably permit the dimension of time-dependent analyses, the integration of genomics and proteomics information would enable one to capture important insights into the complex physical–functional interplays that occur between the combinations of hPTMs present, the factors that bind these regions, the architecture of the recruiting chromatin and the functional readout of histone modifications.

Acknowledgements

We wish to thank A. Cuomo and R. Noberini for critical reading of the manuscript and discussion. TB work is supported by grants from the Giovanni Armenise-Harvard Foundation Career Development Program, the Italian Association for Cancer Research (AIRC) (cod. 11786 and cod. 10000), EPIGEN flagship project Grant (Conv. attuativa-Sottoprog. 6.7) and the Italian Ministry of Health (GR-2009-1580495). We wish to dedicate this article to the memory of the Count Giovanni (Nino) Auletta Armenise, founder of the Giovanni Armenise-Harvard Foundation for Scientific Research.

References

[1] R.D. Kornberg, Chromatin structure: a repeating unit of histones and DNA, Science 184 (1974) 868–871.
[2] K. Luger, A.W. Mader, R.K. Richmond, D.F. Sargent, T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 A resolution, Nature 389 (1997) 251–260.
[3] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[4] P. Tropberger, R. Schneider, Going global: novel histone modifications in the globular domain of H3, Epigenetics 5 (2010) 112–117.
[5] B.D. Strahl, C.D. Allis, The language of covalent histone modifications, Nature 403 (2000) 41–45.
[6] A.J. Bannister, T. Kouzarides, Regulation of chromatin by histone modifications, Cell Res. 21 (2011) 381–395.
[7] O.J. Rando, Combinatorial complexity in chromatin structure and function: revisiting the histone code, Curr. Opin. Genet. Dev. 22 (2012) 148–155.
[8] E.I. Campos, D. Reinberg, Histones: annotating chromatin, Annu. Rev. Genet. 43 (2009) 559–599.
[9] R. Margueron, D. Reinberg, Chromatin structure and the inheritance of epigenetic information, Nat. Rev. Genet. 11 (2010) 285–296.
[10] D.J. Patel, Z. Wang, Readout of epigenetic modifications, Annu. Rev. Biochem. 82 (2013) 81–118.
[11] T. Jenuwein, C.D. Allis, Translating the histone code, Science 293 (2001) 1074–1080.
[12] S.D. Taverna, H. Li, A.J. Ruthenburg, C.D. Allis, D.J. Patel, How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers, Nat. Struct. Mol. Biol. 14 (2007) 1025–1040.
[13] M. Yun, J. Wu, J.L. Workman, B. Li, Readers of histone modifications, Cell Res. 21 (2011) 564–578.
[14] K.L. Yap, M.M. Zhou, Keeping it in the family: diverse histone recognition by conserved structural folds, Crit. Rev. Biochem. Mol. Biol. 45 (2010) 488–505.
[15] S. Khorasanizadeh, Recognition of methylated histones: new twists and variations, Curr. Opin. Struct. Biol. 21 (2011) 744–749.
[16] C. Martin, Y. Zhang, The diverse functions of histone lysine methylation, Nat. Rev. Mol. Cell Biol. 6 (2005) 838–849.
[17] M. Shogren-Knaak, H. Ishii, J.M. Sun, M.J. Pazin, J.R. Davie, C.L. Peterson, Histone H4-K16 acetylation controls chromatin structure and protein interactions, Science 311 (2006) 844–847.
[18] A. Akhtar, P.B. Becker, Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in Drosophila, Mol. Cell 5 (2000) 367–375.
[19] S. Sidoli, L. Cheng, O.N. Jensen, Proteomics in chromatin biology and epigenetics: elucidation of post-translational modifications of histone proteins by mass spectrometry, J. Proteome 75 (2012) 3419–3433.
[20] L.M. Britton, M. Gonzales-Cope, B.M. Zee, B.A. Garcia, Breaking the histone code with quantitative mass spectrometry, Expert Rev. Proteome 8 (2011) 631–643.
[21] M.D. Plazas-Mayorca, B.M. Zee, N.L. Young, I.M. Fingerman, G. LeRoy, S.D. Briggs, B.A. Garcia, One-pot shotgun quantitative mass spectrometry characterization of histones, J. Proteome Res. 8 (2009) 5367–5374.
[22] B.M. Zee, N.L. Young, B.A. Garcia, Quantitative proteomic approaches to studying histone modifications, Curr. Chem. Genomics 5 (2011) 106–114.
[23] B.A. Garcia, Mass spectrometric analysis of histone variants and post-translational modifications, Front. Biosci. (Schol. Ed.) 1 (2009) 142–153.
[24] H.C. Eberl, M. Mann, M. Vermeulen, Quantitative proteomics for epigenetics, Chembiochem 12 (2011) 224–234.
[25] T. Bartke, J. Borgel, P.A. DiMaggio, Proteomics in epigenetics: new perspectives for cancer research, Brief. Funct. Genomics 12 (2013) 205–218.
[26] M. Soldi, A. Cuomo, M. Bremang, T. Bonaldi, Mass spectrometry-based proteomics for the analysis of chromatin structure and dynamics, Int. J. Mol. Sci. 14 (2013) 5402–5431.
[27] Y. Han, B.A. Garcia, Combining genomic and proteomic approaches for epigenetics research, Epigenomics 5 (2013) 439–452.

[28] L. Perez-Burgos, A.H. Peters, S. Opravil, M. Kauer, K. Mechtler, T. Jenuwein, Generation and characterization of methyl-lysine histone antibodies, Methods Enzymol. 376 (2004) 234–254.
[29] Y. Liu, S.D. Taverna, T.L. Muratore, J. Shabanowitz, D.F. Hunt, C.D. Allis, RNAi-dependent H3K27 methylation is required for heterochromatin formation and DNA elimination in Tetrahymena, Genes Dev. 21 (2007) 1530–1545.
[30] I. Bock, A. Dhayalan, S. Kudithipudi, O. Brandt, P. Rathert, A. Jeltsch, Detailed specificity analysis of antibodies binding to modified histone tails with peptide arrays, Epigenetics 6 (2011) 256–263.
[31] P. Cheung, Generation and characterization of antibodies directed against di-modified histones, and comments on antibody and epitope recognition, Methods Enzymol. 376 (2004) 221–234.
[32] M.B. Trelle, O.N. Jensen, Functional proteomics in histone research and epigenetics, Expert Rev. Proteomics 4 (2007) 491–503.
[33] B.A. Garcia, J. Shabanowitz, D.F. Hunt, Characterization of histones and their post-translational modifications by mass spectrometry, Curr. Opin. Chem. Biol. 11 (2007) 66–73.
[34] S.D. Taverna, B.M. Ueberheide, Y. Liu, A.J. Tackett, R.L. Diaz, J. Shabanowitz, B.T. Chait, D.F. Hunt, C.D. Allis, Long-distance combinatorial linkage between methylation and acetylation on histone H3 N termini, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2086–2091.
[35] H.C. Beck, Mass spectrometry in epigenetic research, Methods Mol. Biol. 593 (2010) 263–282.
[36] A. Villar-Garea, A. Imhof, The analysis of histone modifications, Biochim. Biophys. Acta 1764 (2006) 1932–1939.
[37] J.D. Tipton, J.C. Tran, A.D. Catherman, D.R. Ahlf, K.R. Durbin, N.L. Kelleher, Analysis of intact protein isoforms by mass spectrometry, J. Biol. Chem. 286 (2011) 25451–25458.
[38] A.M. Arnaudo, B.A. Garcia, Proteomic characterization of novel histone post-translational modifications, Epigenetics Chromatin 6 (2013) 24.
[39] F. Casadio, X. Lu, S.B. Pollock, G. LeRoy, B.A. Garcia, T.W. Muir, R.G. Roeder, C.D. Allis, H3R42me2a is a histone modification with positive transcriptional effects, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 14894–14899.
[40] M. Tan, H. Luo, S. Lee, F. Jin, J.S. Yang, E. Montellier, T. Buchou, Z. Cheng, S. Rousseaux, N. Rajagopal, Z. Lu, Z. Ye, Q. Zhu, J. Wysocka, Y. Ye, S. Khochbin, B. Ren, Y. Zhao, Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification, Cell 146 (2011) 1016–1028.
[41] Y. Chen, R. Sprung, Y. Tang, H. Ball, B. Sangras, S.C. Kim, J.R. Falck, J. Peng, W. Gu, Y. Zhao, Lysine propionylation and butyrylation are novel post-translational modifications in histones, Mol. Cell. Proteomics 6 (2007) 812–819.
[42] R. Fujiki, W. Hashiba, H. Sekine, A. Yokoyama, T. Chikanishi, S. Ito, Y. Imai, J. Kim, H.H. He, K. Igarashi, J. Kanno, F. Ohtake, H. Kitagawa, R.G. Roeder, M. Brown, S. Kato, GlcNAcylation of histone H2B facilitates its monoubiquitination, Nature 480 (2011) 557–560.
[43] K. Sakabe, Z. Wang, G.W. Hart, Beta-N-acetylglucosamine (O-GlcNAc) is part of the histone code, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 19915–19920.
[44] S. Zhang, K. Roche, H.P. Nasheuer, N.F. Lowndes, Modification of histones by sugar beta-N-acetylglucosamine (GlcNAc) occurs on multiple residues, including histone H3 serine 10, and is cell cycle-regulated, J. Biol. Chem. 286 (2011) 37483–37495.
[45] M. Nikolov, C. Schmidt, H. Urlaub, Quantitative mass spectrometry-based proteomics: an overview, Methods Mol. Biol. 893 (2012) 85–100.
[46] J.J. Pesavento, H. Yang, N.L. Kelleher, C.A. Mizzen, Certain and progressive methylation of histone H4 at lysine 20 during the cell cycle, Mol. Cell. Biol. 28 (2008) 468–486.
[47] D. Bonenfant, H. Towbin, M. Coulot, P. Schindler, D.R. Mueller, J. van Oostrum, Analysis of dynamic changes in post-translational modifications of human histones during cell cycle by mass spectrometry, Mol. Cell. Proteomics 6 (2007) 1917–1932.
[48] A.N. Scharf, K. Meier, V. Seitz, E. Kremmer, A. Brehm, A. Imhof, Monomethylation of lysine 20 on histone H4 facilitates chromatin maturation, Mol. Cell. Biol. 29 (2009) 57–67.
[49] A. Cuomo, S. Moretti, S. Minucci, T. Bonaldi, SILAC-based proteomic analysis to dissect the "histone modification signature" of human breast cancer cells, Amino Acids 41 (2011) 387–399.
[50] S.E. Ong, G. Mittler, M. Mann, Identifying and quantifying in vivo methylation sites by heavy methyl SILAC, Nat. Methods 1 (2004) 119–126.
[51] B.M. Zee, R.S. Levin, B. Xu, G. LeRoy, N.S. Wingreen, B.A. Garcia, In vivo residue-specific histone methylation dynamics, J. Biol. Chem. 285 (2010) 3341–3350.
[52] X.J. Cao, B.M. Zee, B.A. Garcia, Heavy methyl-SILAC labeling coupled with liquid chromatography and high-resolution mass spectrometry to study the dynamics of site-specific histone methylation, Methods Mol. Biol. 977 (2013) 299–313.
[53] B.D. Fodor, S. Kubicek, M. Yonezawa, R.J. O'Sullivan, R. Sengupta, L. Perez-Burgos, S. Opravil, K. Mechtler, G. Schotta, T. Jenuwein, Jmjd2b antagonizes H3K9 trimethylation at pericentric heterochromatin in mammalian cells, Genes Dev. 20 (2006) 1557–1562.
[54] S.M. Sweet, M. Li, P.M. Thomas, K.R. Durbin, N.L. Kelleher, Kinetics of re-establishing H3K79 methylation marks in global human chromatin, J. Biol. Chem. 285 (2010) 32778–32786.
[55] Y. Zheng, S.M. Sweet, R. Popovic, E. Martinez-Garcia, J.D. Tipton, P.M. Thomas, J.D. Licht, N.L. Kelleher, Total kinetic analysis reveals how combinatorial methylation patterns are established on lysines 27 and 36 of histone H3, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 13549–13554.
[56] S.E. Peach, E.L. Rudomin, N.D. Udeshi, S.A. Carr, J.D. Jaffe, Quantitative assessment of chromatin immunoprecipitation grade antibodies directed against histone modifications reveals patterns of co-occurring marks on histone protein molecules, Mol. Cell. Proteomics 11 (2012) 128–137.
[57] B.A. Garcia, J.J. Pesavento, C.A. Mizzen, N.L. Kelleher, Pervasive combinatorial modification of histone H3 in human cells, Nat. Methods 4 (2007) 487–489.
[58] N.L. Young, P.A. DiMaggio, M.D. Plazas-Mayorca, R.C. Baliban, C.A. Floudas, B.A. Garcia, High throughput characterization of combinatorial histone codes, Mol. Cell. Proteomics 8 (2009) 2266–2284.
[59] J.J. Pesavento, Y.B. Kim, G.K. Taylor, N.L. Kelleher, Shotgun annotation of histone modifications: a new approach for streamlined characterization of proteins by top down mass spectrometry, J. Am. Chem. Soc. 126 (2004) 3386–3387.
[60] L.M. Smith, N.L. Kelleher, Proteoform: a single term describing protein complexity, Nat. Methods 10 (2013) 186–187.
[61] K.R. Durbin, J.C. Tran, L. Zamdborg, S.M. Sweet, A.D. Catherman, J.E. Lee, M. Li, J. F. Kellie, N.L. Kelleher, Intact mass detection, interpretation, and visualization to automate top-down proteomics on a large scale, Proteomics 10 (2010) 3589–3597.
[62] C.E. Thomas, N.L. Kelleher, C.A. Mizzen, Mass spectrometric characterization of human histone H3: a bird's eye view, J. Proteome Res. 5 (2006) 240–247.
[63] M.T. Boyne II, J.J. Pesavento, C.A. Mizzen, N.L. Kelleher, Precise characterization of human histones in the H2A gene family by top down mass spectrometry, J. Proteome Res. 5 (2006) 248–253.
[64] N. Siuti, M.J. Roth, C.A. Mizzen, N.L. Kelleher, J.J. Pesavento, Gene-specific characterization of human histone H2B by electron capture dissociation, J. Proteome Res. 5 (2006) 233–239.
[65] J.J. Pesavento, C.A. Mizzen, N.L. Kelleher, Quantitative analysis of modified proteins and their positional isomers by tandem mass spectrometry: human histone H4, Anal. Chem. 78 (2006) 4271–4280.
[66] Y. Zheng, P.M. Thomas, N.L. Kelleher, Measurement of acetylation turnover at distinct lysines in human histones identifies long-lived acetylation sites, Nat. Commun. 4 (2013) 2203.
[67] J.J. Pesavento, C.R. Bullock, R.D. LeDuc, C.A. Mizzen, N.L. Kelleher, Combinatorial modification of human histone H4 quantitated by two-dimensional liquid chromatography coupled with top down mass spectrometry, J. Biol. Chem. 283 (2008) 14927–14937.
[68] H.R. Jung, S. Sidoli, S. Haldbo, R.R. Sprenger, V. Schwammle, D. Pasini, K. Helin, O.N. Jensen, Precision mapping of coexisting modifications in histone H3 tails from embryonic stem cells by ETD-MS/MS, Anal. Chem. 85 (2013) 8232–8239.
[69] P.A. DiMaggio Jr., N.L. Young, R.C. Baliban, B.A. Garcia, C.A. Floudas, A mixed integer linear optimization framework for the identification and quantification of targeted post-translational modifications of highly modified proteins using multiplexed electron transfer dissociation tandem mass spectrometry, Mol. Cell. Proteomics 8 (2009) 2527–2543.
[70] A.M. Frank, J.J. Pesavento, C.A. Mizzen, N.L. Kelleher, P.A. Pevzner, Interpreting top-down mass spectra using spectral alignment, Anal. Chem. 80 (2008) 2499–2505.
[71] S. Guan, A.L. Burlingame, Data processing algorithms for analysis of high resolution MSMS spectra of peptides with complex patterns of posttranslational modifications, Mol. Cell. Proteomics 9 (2010) 804–810.
[72] R.J. Rose, E. Damoc, E. Denisov, A. Makarov, A.J. Heck, High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies, Nat. Methods 9 (2012) 1084–1086.
[73] X. Shi, I. Kachirskaia, K.L. Walter, J.H. Kuo, A. Lake, F. Davrazou, S.M. Chan, D.G. Martin, I.M. Fingerman, S.D. Briggs, L. Howe, P.J. Utz, T.G. Kutateladze, A.A. Lugovskoy, M.T. Bedford, O. Gozani, Proteome-wide analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36, J. Biol. Chem. 282 (2007) 2450–2455.
[74] P. Filippakopoulos, S. Picaud, M. Mangos, T. Keates, J.P. Lambert, D. Barsyte-Lovejoy, I. Felletar, R. Volkmer, S. Muller, T. Pawson, A.C. Gingras, C.H. Arrowsmith, S. Knapp, Histone recognition and large-scale structural analysis of the human bromodomain family, Cell 149 (2012) 214–231.
[75] Y. Yang, Y. Lu, A. Espejo, J. Wu, W. Xu, S. Liang, M.T. Bedford, TDRD3 is an effector molecule for arginine-methylated histone marks, Mol. Cell 40 (2010) 1016–1023.
[76] S. Maurer-Stroh, N.J. Dickens, L. Hughes-Davies, T. Kouzarides, F. Eisenhaber, C.P. Ponting, The Tudor domain 'Royal Family': Tudor, plant Agenet, Chromo, PWWP and MBT domains, Trends Biochem. Sci. 28 (2003) 69–74.
[77] J. Wysocka, T. Swigut, T.A. Milne, Y. Dou, X. Zhang, A.L. Burlingame, R.G. Roeder, A.H. Brivanlou, C.D. Allis, WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development, Cell 121 (2005) 859–872.
[78] J. Wysocka, T. Swigut, H. Xiao, T.A. Milne, S.Y. Kwon, J. Landry, M. Kauer, A.J. Tackett, B.T. Chait, P. Badenhorst, C. Wu, C.D. Allis, A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling, Nature 442 (2006) 86–90.
[79] M. Vermeulen, K.W. Mulder, S. Denissov, W.W. Pijnappel, F.M. van Schaik, R.A. Varier, M.P. Baltissen, H.G. Stunnenberg, M. Mann, H.T. Timmers, Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4, Cell 131 (2007) 58–69.
[80] M. Vermeulen, H.C. Eberl, F. Matarese, H. Marks, S. Denissov, F. Butter, K.K. Lee, J.V. Olsen, A.A. Hyman, H.G. Stunnenberg, M. Mann, Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers, Cell 142 (2010) 967–980.
[81] X. Li, E.A. Foley, K.R. Molloy, Y. Li, B.T. Chait, T.M. Kapoor, Quantitative chemical proteomics approach to identify post-translational modification-mediated protein–protein interactions, J. Am. Chem. Soc. 134 (2012) 1982–1985.
[82] T.M. Spektor, J.C. Rice, Identification and characterization of posttranslational modification-specific binding proteins in vivo by mammalian tethered catalysis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14808–14813.
[83] T. Bartke, M. Vermeulen, B. Xhemalce, S.C. Robson, M. Mann, T. Kouzarides, Nucleosome-interacting proteins regulated by DNA and histone methylation, Cell 143 (2010) 470–484.
[84] M. Nikolov, A. Stutzer, K. Mosch, A. Krasauskas, S. Soeroes, H. Stark, H. Urlaub, W. Fischle, Chromatin affinity purification and quantitative mass spectrometry

defining the interactome of histone modification patterns, Mol. Cell. Proteomics 10 (2011) (M110 005371).
[85] E. Shema-Yaacoby, M. Nikolov, M. Haj-Yahya, P. Siman, E. Allemand, Y. Yamaguchi, C. Muchardt, H. Urlaub, A. Brik, M. Oren, W. Fischle, Systematic identification of proteins binding to chromatin-embedded ubiquitylated H2B reveals recruitment of SWI/SNF to regulate transcription, Cell Rep. 4 (2013) 601–608.
[86] H.R. Jung, D. Pasini, K. Helin, O.N. Jensen, Quantitative mass spectrometry of histones H3.2 and H3.3 in Suz12-deficient mouse embryonic stem cells reveals distinct, dynamic post-translational modifications at Lys-27 and Lys-36, Mol. Cell. Proteomics 9 (2010) 838–850.
[87] J. Dejardin, R.E. Kingston, Purification of proteins associated with specific genomic Loci, Cell 136 (2009) 175–186.
[88] J.M. Antao, J.M. Mason, J. Dejardin, R.E. Kingston, Protein landscape at *Drosophila melanogaster* telomere-associated sequence repeats, Mol. Cell. Biol. 32 (2012) 2170–2182.
[89] A. Unnikrishnan, P.R. Gafken, T. Tsukiyama, Dynamic changes in histone acetylation regulate origins of DNA replication, Nat. Struct. Mol. Biol. 17 (2010) 430–437.
[90] S.D. Byrum, A. Raman, S.D. Taverna, A.J. Tackett, ChAP–MS: a method for identification of proteins and histone posttranslational modifications at a single genomic locus, Cell Rep. 2 (2012) 198–205.
[91] S.D. Byrum, S.D. Taverna, A.J. Tackett, Purification of a specific native genomic locus for proteomic analysis, Nucleic Acids Res. 41 (2013) e195.
[92] A. Barski, S. Cuddapah, K. Cui, T.Y. Roh, D.E. Schones, Z. Wang, G. Wei, I. Chepelev, K. Zhao, High-resolution profiling of histone methylations in the human genome, Cell 129 (2007) 823–837.
[93] D.S. Johnson, A. Mortazavi, R.M. Myers, B. Wold, Genome-wide mapping of in vivo protein–DNA interactions, Science 316 (2007) 1497–1502.
[94] P.J. Park, Epigenetics meets next-generation sequencing, Epigenetics 3 (2008) 318–321.
[95] P.J. Park, ChIP-seq: advantages and challenges of a maturing technology, Nat. Rev. Genet. 10 (2009) 669–680.
[96] G. Robertson, M. Hirst, M. Bainbridge, M. Bilenky, Y. Zhao, T. Zeng, G. Euskirchen, B. Bernier, R. Varhol, A. Delaney, N. Thiessen, O.L. Griffith, A. He, M. Marra, M. Snyder, S. Jones, Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing, Nat. Methods 4 (2007) 651–657.
[97] T.S. Mikkelsen, M. Ku, D.B. Jaffe, B. Issac, E. Lieberman, G. Giannoukos, P. Alvarez, W. Brockman, T.K. Kim, R.P. Koche, W. Lee, E. Mendenhall, A. O'Donovan, A. Presser, C. Russ, X. Xie, A. Meissner, M. Wernig, R. Jaenisch, C. Nusbaum, E.S. Lander, B.E. Bernstein, Genome-wide maps of chromatin state in pluripotent and lineage-committed cells, Nature 448 (2007) 553–560.
[98] J.P. Lambert, L. Mitchell, A. Rudner, K. Baetz, D. Figeys, A novel proteomics approach for the discovery of chromatin-associated protein networks, Mol. Cell. Proteomics 8 (2009) 870–882.
[99] J.P. Lambert, J. Fillingham, M. Siahbazi, J. Greenblatt, K. Baetz, D. Figeys, Defining the budding yeast chromatin-associated interactome, Mol. Syst. Biol. 6 (2010) 448.
[100] M. Soldi, T. Bonaldi, The proteomic investigation of chromatin functional domains reveals novel synergisms among distinct heterochromatin components, Mol. Cell. Proteomics 12 (2013) 764–780.
[101] C.I. Wang, A.A. Alekseyenko, G. LeRoy, A.E. Elia, A.A. Gorchakov, L.M. Britton, S.J. Elledge, P.V. Kharchenko, B.A. Garcia, M.I. Kuroda, Chromatin proteins captured by ChIP-mass spectrometry are linked to dosage compensation in Drosophila, Nat. Struct. Mol. Biol. 20 (2013) 202–209.
[102] E. Larschan, A.A. Alekseyenko, A.A. Gortchakov, S. Peng, B. Li, P. Yang, J.L. Workman, P.J. Park, M.I. Kuroda, MSL complex is attracted to genes marked by H3K36 trimethylation using a sequence-independent mechanism, Mol. Cell 28 (2007) 121–133.
[103] O. Bell, T. Conrad, J. Kind, C. Wirbelauer, A. Akhtar, D. Schubeler, Transcription-coupled methylation of histone H3 at lysine 36 regulates dosage compensation by enhancing recruitment of the MSL complex in Drosophila melanogaster, Mol. Cell. Biol. 28 (2008) 3401–3409.
[104] M.E. Gelbart, E. Larschan, S. Peng, P.J. Park, M.I. Kuroda, Drosophila MSL complex globally acetylates H4K16 on the male X chromosome for dosage compensation, Nat. Struct. Mol. Biol. 16 (2009) 825–832.
[105] D.K. Pokholok, C.T. Harbison, S. Levine, M. Cole, N.M. Hannett, T.I. Lee, G.W. Bell, K. Walker, P.A. Rolfe, E. Herbolsheimer, J. Zeitlinger, F. Lewitter, D.K. Gifford, R.A. Young, Genome-wide map of nucleosome acetylation and methylation in yeast, Cell 122 (2005) 517–527.
[106] S.E. Ong, B. Blagoev, I. Kratchmarova, D.B. Kristensen, H. Steen, A. Pandey, M. Mann, Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics, Mol. Cell. Proteomics 1 (2002) 376–386.
[107] S.E. Ong, M. Mann, A practical recipe for stable isotope labeling by amino acids in cell culture SILAC, Nat. Protoc. 1 (2006) 2650–2660.
[108] S.E. Ong, M. Mann, Stable isotope labeling by amino acids in cell culture for quantitative proteomics, Methods Mol. Biol. 359 (2007) 37–52.
[109] M. Soldi, T. Bonaldi, The ChroP approach combines ChIP and mass spectrometry to dissect locus-specific proteomic landscapes of chromatin, Vis. Exp. (2014) e51220, http://dx.doi.org/10.3791/51220 (in press).
[110] D.N. Perkins, D.J. Pappin, D.M. Creasy, J.S. Cottrell, Probability-based protein identification by searching sequence databases using mass spectrometry data, Electrophoresis 20 (1999) 3551–3567.
[111] J. Cox, N. Neuhauser, A. Michalski, R.A. Scheltema, J.V. Olsen, M. Mann, Andromeda: a peptide search engine integrated into the MaxQuant environment, J. Proteome Res. 10 (2011) 1794–1805.
[112] T.S. Furey, ChIP-seq and beyond: new and improved methodologies to detect and characterize protein–DNA interactions, Nat. Rev. Genet. 13 (2012) 840–852.
[113] D. Doenecke, Cesium chloride gradients of chromatin after treatment with micrococcal nuclease, Cell 8 (1976) 59–64.
[114] H. Noll, M. Noll, Sucrose gradient techniques and applications to nucleosome structure, Methods Enzymol. 170 (1989) 55–116.
[115] A.W. Thorne, F.A. Myers, T.R. Hebbes, Native chromatin immunoprecipitation, Methods Mol. Biol. 287 (2004) 21–44.
[116] P. Voigt, G. LeRoy, W.J. Drury III, B.M. Zee, J. Son, D.B. Beck, N.L. Young, B.A. Garcia, D. Reinberg, Asymmetrically modified nucleosomes, Cell 151 (2012) 181–193.
[117] N.C. Hubner, A.W. Bird, J. Cox, B. Splettstoesser, P. Bandilla, I. Poser, A. Hyman, M. Mann, Quantitative proteomics combined with BAC TransgeneOmics reveals in vivo protein interactions, J. Cell Biol. 189 (2010) 739–754.
[118] N.C. Hubner, M. Mann, Extracting gene function from protein–protein interactions using Quantitative BAC InteraCtomics QUBIC, Methods 53 (2011) 453–459.
[119] S. Driegen, R. Ferreira, A. van Zon, J. Strouboulis, M. Jaegle, F. Grosveld, S. Philipsen, D. Meijer, A generic tool for biotinylation of tagged proteins in transgenic mice, Transgenic Res. 14 (2005) 477–482.
[120] G. Strubbe, C. Popp, A. Schmidt, A. Pauli, L. Ringrose, C. Beisel, R. Paro, Polycomb purification by in vivo biotinylation tagging reveals cohesin and Trithorax group proteins as interaction partners, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5572–5577.
[121] J. Kim, A.B. Cantor, S.H. Orkin, J. Wang, Use of in vivo biotinylation to study protein–protein and protein–DNA interactions in mouse embryonic stem cells, Nat. Protoc. 4 (2009) 506–517.
[122] P.N. Lau, P. Cheung, Elucidating combinatorial histone modifications and crosstalks by coupling histone-modifying enzyme with biotin ligase activity, Nucleic Acids Res. 41 (2012) e49.
[123] T. Kocher, R. Swart, K. Mechtler, Ultra-high-pressure RPLC hyphenated to an LTQ-Orbitrap Velos reveals a linear relation between peak capacity and number of identified peptides, Anal. Chem. 83 (2011) 2699–2704.
[124] N. Nagaraj, N.A. Kulak, J. Cox, N. Neuhauser, K. Mayr, O. Hoerning, O. Vorm, M. Mann, System-wide perturbation analysis with nearly complete coverage of the yeast proteome by single-shot ultra HPLC runs on a bench top Orbitrap, Mol. Cell. Proteomics 11 (2012) (M111 013722).
[125] A. Goffeau, B.G. Barrell, H. Bussey, R.W. Davis, B. Dujon, H. Feldmann, F. Galibert, J.D. Hoheisel, C. Jacq, M. Johnston, E.J. Louis, H.W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, S.G. Oliver, Life with 6000 genes, Science 274 (546) (1996) 563–567.
[126] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E. Charpentier, A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science 337 (2012) 816–821.
[127] L. Cong, F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P.D. Hsu, X. Wu, W. Jiang, L.A. Marraffini, F. Zhang, Multiplex genome engineering using CRISPR/Cas systems, Science 339 (2013) 819–823.
[128] J.E. DiCarlo, J.E. Norville, P. Mali, X. Rios, J. Aach, G.M. Church, Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems, Nucleic Acids Res. 41 (2013) 4336–4343.
[129] T. Gaj, C.A. Gersbach, C.F. Barbas III, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, Trends Biotechnol. 31 (2013) 397–405.
[130] G. Liang, Y. Zhang, Embryonic stem cell and induced pluripotent stem cell: an epigenetic perspective, Cell Res. 23 (2013) 49–69.
[131] S. Melcer, E. Meshorer, Chromatin plasticity in pluripotent cells, Essays Biochem. 48 (2010) 245–262.
[132] G. Natoli, Maintaining cell identity through global control of genomic organization, Immunity 33 (2010) 12–24.
[133] A. Tarakhovsky, Logic of the inflammation-associated transcriptional response, Adv. Immunol. 119 (2013) 107–133.
[134] J.V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, Cell 127 (2006) 635–648.
[135] H.C. Eberl, C.G. Spruijt, C.D. Kelstrup, M. Vermeulen, M. Mann, A map of general and specialized chromatin readers in mouse tissues generated by label-free interaction proteomics, Mol. Cell 49 (2013) 368–378.
[136] P. Picotti, B. Bodenmiller, L.N. Mueller, B. Domon, R. Aebersold, Full dynamic range proteome analysis of *S. cerevisiae* by targeted proteomics, Cell 138 (2009) 795–806.
[137] L. Anderson, C.L. Hunter, Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins, Mol. Cell. Proteomics 5 (2006) 573–588.
[138] P. Picotti, R. Aebersold, Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions, Nat. Methods 9 (2012) 555–566.
[139] B. Domon, Considerations on selected reaction monitoring experiments: implications for the selectivity and accuracy of measurements, Proteomics Clin. Appl. 6 (2012) 609–614.
[140] P. Picotti, O. Rinner, R. Stallmach, F. Dautel, T. Farrah, B. Domon, H. Wenschuh, R. Aebersold, High-throughput generation of selected reaction-monitoring assays for proteins and proteomes, Nat. Methods 7 (2010) 43–46.
[141] S. Gallien, E. Duriez, B. Domon, Selected reaction monitoring applied to proteomics, J. Mass Spectrom. 46 (2011) 298–312.
[142] S. Gallien, E. Duriez, C. Crone, M. Kellmann, T. Moehring, B. Domon, Targeted proteomic quantification on quadrupole-orbitrap mass spectrometer, Mol. Cell. Proteomics 11 (2012) 1709–1723.
[143] S. Gallien, A. Bourmaud, S.Y. Kim, B. Domon, Technical considerations for large-scale parallel reaction monitoring analysis, J. Proteomics. 100C (2014) 147–159.
[144] A.C. Peterson, J.D. Russell, D.J. Bailey, M.S. Westphall, J.J. Coon, Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics, Mol. Cell. Proteomics 11 (2012) 1475–1488.
[145] J.C. Tran, L. Zamdborg, D.R. Ahlf, J.E. Lee, A.D. Catherman, K.R. Durbin, J.D. Tipton, A. Vellaichamy, J.F. Kellie, M. Li, C. Wu, S.M. Sweet, B.P. Early, N. Siuti, R.D. LeDuc, P.D.

Compton, P.M. Thomas, N.L. Kelleher, Mapping intact protein isoforms in discovery mode using top-down proteomics, Nature 480 (2011) 254–258.
[146] J.C. Tran, A.A. Doucette, Gel-eluted liquid fraction entrapment electrophoresis: an electrophoretic method for broad molecular weight range proteome separation, Anal. Chem. 80 (2008) 1568–1573.
[147] J.F. Kellie, A.D. Catherman, K.R. Durbin, J.C. Tran, J.D. Tipton, J.L. Norris, C.E. Witkowski II, P.M. Thomas, N.L. Kelleher, Robust analysis of the yeast proteome under 50 kDa by molecular-mass-based fractionation and top-down mass spectrometry, Anal. Chem. 84 (2012) 209–215.
[148] F. Lanucara, C.E. Eyers, Top-down mass spectrometry for the analysis of combinatorial post-translational modifications, Mass Spectrom. Rev. 32 (2013) 27–42.
[149] L. Zamdborg, R.D. LeDuc, K.J. Glowacz, Y.B. Kim, V. Viswanathan, I.T. Spaulding, B.P. Early, E.J. Bluhm, S. Babai, N.L. Kelleher, ProSight PTM 2.0: improved protein identification and characterization for top down mass spectrometry, Nucleic Acids Res. 35 (2007) W701–W706.
[150] N.M. Karabacak, L. Li, A. Tiwari, L.J. Hayward, P. Hong, M.L. Easterling, J.N. Agar, Sensitive and specific identification of wild type and variant proteins from 8 to 669 kDa using top-down mass spectrometry, Mol. Cell. Proteomics 8 (2009) 846–856.
[151] D. Karolchik, G.P. Barber, J. Casper, H. Clawson, M.S. Cline, M. Diekhans, T.R. Dreszer, P.A. Fujita, L. Guruvadoo, M. Haeussler, R.A. Harte, S. Heitner, A.S. Hinrichs, K. Learned, B.T. Lee, C.H. Li, B.J. Raney, B. Rhead, K.R. Rosenbloom, C.A. Sloan, M.L. Speir, A.S. Zweig, D. Haussler, R.M. Kuhn, W.J. Kent, The UCSC Genome Browser database: 2014 update, Nucleic Acids Res. 42 (2014) D764–D770.
[152] P.V. Kharchenko, A.A. Alekseyenko, Y.B. Schwartz, A. Minoda, N.C. Riddle, J. Ernst, P.J. Sabo, E. Larschan, A.A. Gorchakov, T. Gu, D. Linder-Basso, A. Plachetka, G. Shanower, M.Y. Tolstorukov, L.J. Luquette, R. Xi, Y.L. Jung, R.W. Park, E.P. Bishop, T.K. Canfield, R. Sandstrom, R.E. Thurman, D.M. MacAlpine, J.A. Stamatoyannopoulos, M. Kellis, S.C. Elgin, M.I. Kuroda, V. Pirrotta, G.H. Karpen, P.J. Park, Comprehensive analysis of the chromatin landscape in *Drosophila melanogaster*, Nature 471 (2011) 480–485.