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Models and tools used to study epigenetics

Mass spectrometry analysis of histone post translational modifications

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Histone proteins play a central role in the dynamic structure of chromatin within the cell nucleus. The post translational modifications of histones can perturb chromatin contacts and the recruitment of non-histone proteins to chromatin. Alterations in the chromatin structure in which DNA is packaged influences the assembly of enzyme complexes that subsequently manipulate DNA. Therefore, such modifications may affect a wide range of biological processes which are potentially epigenetically inherited. The nature of histone marks and their importance in the epigenetic regulation of cellular functions has made them a prime candidate for study in both disease and drug discovery. The development of analytical tools that enable the identification and quantification of histone post translational modifications is therefore of significant interest. Mass spectrometry is a powerful tool for the global, unbiased, quantitative analysis of histone post translational modifications. Mass spectrometry allows the combinatorial nature of histone post translational modifications to be explored and has revolutionised the ability to study the histone code.

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Introduction

Epigenetics describes the heritable changes in gene expression without a change in the DNA sequence itself and includes; histone post translational modifications, DNA methylation and non-coding RNAs. It is important to note that these three mechanisms do not act independently to exert their function, but instead act in concert, enforcing and repressing other epigenetic marks [1]. This review will focus on one of these mechanisms: histone post translational modifications.

Histone modifications and gene regulation

The nucleosome, the fundamental structural unit of chromatin is made up from a complex of 8 histone proteins which has coiled around it approximately 147 bp of DNA [2]. The histone octamer contains 2 copies of each of the 4 histone proteins: H2A, H2B, H3 and H4. Importantly these histone proteins can have post translational modifications (PTMs) which are focused at the N-terminal tail. Although some modifications are present in the globular and C-terminal regions, these are far sparser than the dense collection of modifications at the N-terminal tail [3]. These chemical modifications affect not only the structure of chromatin, but also the recruitment of effector proteins, and therefore influence gene expression. This in effect is the basis of the histone code [4]. A vast array of histone PTMs have been identified, including, but not limited to mono, di and trimethylation, acetylation, phosphorylation, SUMOylation, crotonylation and ubiquitination [4–7]. The role of histone acetylation in the activational control of the HO gene in yeast

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has been well characterized and is a good example of the role that histone modifications play in gene expression. The binding of SWI5 to an upstream activating sequence within the DNA acts as a binding site for the SAGA protein complex, a subunit of which is the histone acetyltransferase (GCN5). Recruitment of the SAGA complex results in the acetylation of histone H4. This acetylation changes the overall charge of the histone proteins causing the ionic bonds between the negatively charged phosphate groups on the DNA backbone and the positive charge from the histone proteins to weaken resulting in a more open conformation of chromatin [8–10]. In addition, following the histone acetylation, the SWI/SNF complex can bind via its bromodomain to the acetylated H4K8 [11–13]. The SWI/SNF complex with the action of the ATPase domain effectively slides nucleosomes along around 36 bp of linker DNA, causing new areas of DNA to be exposed, leading to the recruitment of the transcription factor TFIID from the pre-initiation complex (PIC). The recruitment of TFIID is dependent upon the state of histone modifications around the TATA box (the genomic region to which the TBP domain of TFIID binds). The subunit TAFII250 (TAF1) contains two bromodomains that bind to H4K5ac, K8ac, K12ac, and K16ac with micromolar affinity. This binding is both sequence and modification specific [14]. The binding of TAF3 to histone H3K4me3 acts along with other histone modifications to selectively anchor TFIID [15]. The binding of which allows the assembly of the PIC and therefore the start of transcription.

The combinatorial complexity and amount of information that can be contained within histones is vast. A good example of this is lysine9 on histone H3 which has been identified with a range of modifications including; mono, di, tri-methylation, acetylation, ubiquitination, SUMOylation, crotonylation and recently 2-hydroxyisobutyrylation [5,7,16,17]. This lysine is just one of numerous sites that can be modified on histone H3, the majority of which can also be modified in more than one way. Combined with the presence of asynchronous histone modifications (modifications present on only one of two H3 histone proteins in the same nucleosome) the full extent of the combinatorial complexity becomes apparent [18].

Identification of histone PTMs using antibody based techniques

Antibody based techniques, widely used for studying histone PTMs, are enabled by the creation of modification specific antibodies. These antibodies are not only specific to a particular chemical modification but are also sequence specific. Antibody based techniques such as western blots and dot blot analysis have been used not only to provide identification of histone modifications but also semi-quantitative data. These techniques have been used to probe the role of histone modifications in a multitude of systems and have forwarded the field of epigenetics enormously [14–16]. Antibodies provide a very sensitive, high throughput method for the detection of histone PTMs and have been used to associate histone PTMs with cancer [19], immunity [20] and in a wide range of other fields, beyond the scope of this review.

However, as with all techniques, there are a number of limitations with respect to antibody based techniques for probing histone modifications, including problems associated with selectivity, epitope occlusion and cross reactivity [21–24]. Moreover, antibody based approaches usually can only detect one modification at a time and are not directly able to identify new or novel histone PTMs.

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) allows specifically modified histones to be immunoprecipitated along with its corresponding DNA sequence, thus allowing the mapping of certain histone modifications to their genomic location. This technique is often used in conjunction with next generation sequencing tools to perform ChIP-seq or with microarray technology in ChIP-chip to provide a high throughput method of linking histone PTMs to genomic location [24]. These techniques enable researchers to probe the epigenome and have enabled significant advances in the field of chromatin and epigenetics research. The current technologies have been reviewed by Collas [24].

Identification and characterisation of histone PTMs using mass spectrometry

Mass spectrometry (MS) represents an orthologous pathway by which histone modifications can be identified and is often considered as the gold standard for protein identification and quantification [25]. Mass spectrometers measure the mass to charge ratio (m/z) of ions in the gas phase. In the case of proteins and peptides, their ionization and delivery into the gas phase is achieved by alternative MS ion sources: (1) matrix assisted laser desorption ionization (MALDI) or (2) electrospray ionization (ESI) via an online high performance liquid chromatography system (HPLC). The output of m/z values from the MS can be used to determine very accurately the mass of a protein or peptide (precursor mass). This information is then combined with tandem MS (MS/MS) to enable unambiguous identification of the peptide sequence and more importantly the precise location of any PTMs that might be present. MS/MS is performed by isolating a specific precursor ion and inducing its fragmentation into a series of product ions (see Fig. 1). The m/z values for these ions are measured and mapped to protein sequences in a database to provide identification. Moreover, the position of the amino acid that has been modified can be elucidated (see Fig. 1b).

Fragmentation for tandem MS is commonly performed using collision induced dissociation (CID). In CID, the isolated precursor ions are, depending on the type of MS either directed