Comprehensive Characterization of Antibodies Directed towards Epigenetic Histone-Modifications

A. Masch*, U. Reimer[ɣ], M. Schutkowski*

*Institute of Biochemistry and Biotechnology, Department of Enzymology, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Str. 3, 06120 Halle, Germany.

[ɣ] JPT Peptide Technologies, Volmerstrasse 5 12489 Berlin, Germany

The Histone Code is important to regulate the transcription of genetic information from DNA. Numerous enzymes and protein domains modify and recognize the Histone Code as writers/erasers or readers, respectively. Reliable tools are needed for the specific characterization of Histone modifications at a given time in order to understand the biological implications of this code. For this, modification specific antibodies are frequently used. However, the binding-specificity of such antibodies is in many cases different from what is claimed. Here we present comprehensive peptide microarrays representing the sequence diversity and several potential modifications for the main histone isoforms. These peptide microarrays are perfectly suited to determine both subsite specificity of antibodies and cross-reactivity with other histone marks.

Introduction

Histones are the protein component of the nucleosome which is the package unit for the DNA which is wrapped around a histone octamer formed by dimers of Histones H2A, H2B, H3 and H4. The N-terminal and partly the C-terminal tails of each histone are not structured and well accessible for enzymes and binding partners. Therefore, it is not surprising that these tails are heavily modified by phosphorylations, acylations, methylations, and glycosylations. The modifications of the histone tails within a nucleosome determine specifically the state of the DNA sequence around this nucleosome like gene transcription activity or DNA repair. Antibodies directed against these so called epigenetic marks are widely used for uncovering the Histone Code for each nucleosome (**Ch**romatin **I**mmune **P**recipitation = ChIP). Nevertheless, exact knowledge of the recognition of the respective epigenetic mark in combination with other modifications in the surrounding of this epitope often is not available. Additionally, cross-reactivity of such antibodies with other modifications would result in false positive readouts during ChIP experiments. Therefore we developed a technology for extremely efficient quality control of antibodies directed against epigenetic marks in histone proteins.

Results

Library Design: The aim of the library is the complete coverage of sequence diversity of Histones and to present all potential modifications at Lysine, Arginine, Serine, Threonine and Tyrosine. Systematically, peptide scans of 20mer peptides with an overlap of 10 amino acids were generated for all available isoforms from Uniprot for the Histones H2A (15 isoforms), H2B (15), H3 (5) and H4 (1). We added single modifications at lysine (Kac, Kme1, Kme2, Kme3, Kbut, Kprop, Kmal, Ksuc), arginine (Rme1, Rme2a, Rme2s, Cit) and phosphorylations at threonine, serine and tyrosine, resulting in 2819 peptides. In a second, knowledge-based part of the library, 450 peptides derived mostly from the histone tails with multiple modifications (up to six modifications within one peptide which were experimentally verified) were generated. Additionally, 604 peptides covering the isoforms of Histone H1 and experimentally detected post-translational modifications were added resulting in a total of 3873 peptides presented in triplicates on the Histone Code Microarray.

Microarray experiments: Histone Code Microarrays were treated with anti-Lys(Succ) or anti-Lys(Ac) antibodies for one hour at room temperature. After washings a fluorescently labelled anti-IgG antibody was added. Subsequent to washings and drying fluorescence images were generated using an Axon4000B scanner.

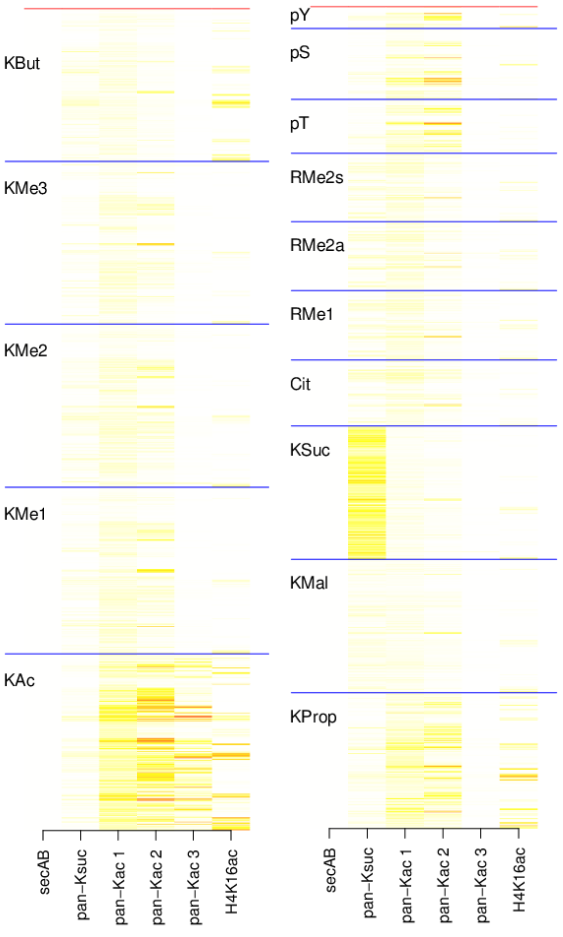

Figure 1. Heatmap of signal intensities for different Histone-specific antibodies. Peptides with indicated single modifications are shown on the y-axis. Signal intensity ranges from white (noise) to red (max signal). The map is divided into two parts and each lane represents one antibody

Histone Code Peptide Microarrays

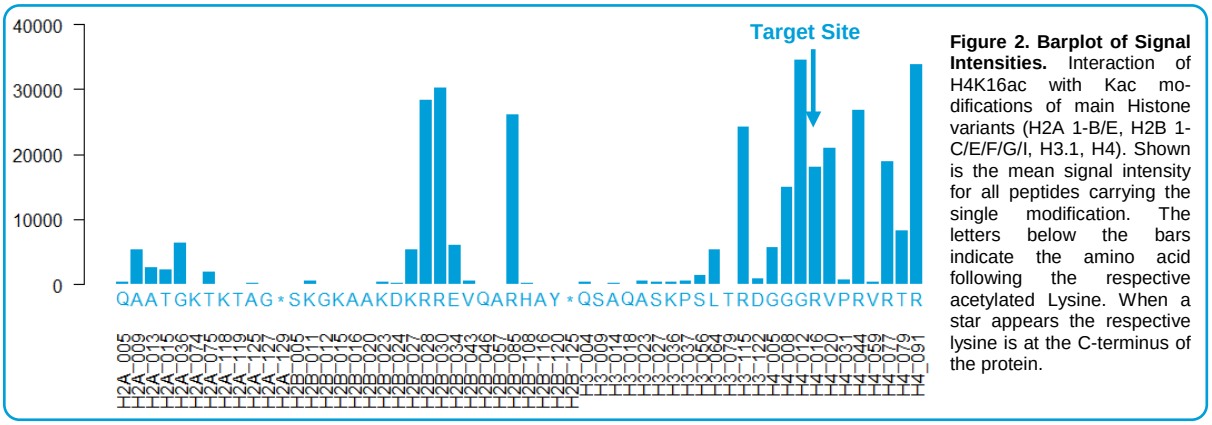

Figure 2. Barplot of Signal Intensities. Interaction of H4K16ac with Kac modifications of main Histone variants (H2A 1-B/E, H2B 1-C/E/F/G/I, H3.1, H4). Shown is the mean signal intensity for all peptides carrying the single modification. The letters below the bars indicate the amino acid following the respective acetylated Lysine. When a star appears the respective lysine is at the C-terminus of the protein.

Recorded signal intensities for each individual antibody towards histone peptides with single modifications are shown in **Figure 1**. No signals are observed for the control incubation with a mixture of the secondary antibodies. The second column shows signals for a pan-specific antibody directed against succinylated lysines. Signals are almost exclusively observed for nearly all of the presented succinylated peptides indicating both, a high specificity of this antibody for the chemical nature of the acyl moiety on the lysine side chain, and no subsite specificity regarding the peptide sequence. Recently, we profiled pan-specific anti-Lys(Ac)-antibodies using acetylome peptide microarrays displaying more than 6500 human peptides in acetylated and non-acetylated form (1). We profiled these antibodies using the Histone Code Microarray (Figure1 lanes 3-5). In contrast to the anti-Lys(Succ)-antibody the analysed anti-Lys(Ac) antibodies recognize only a subset of peptide with Kac. More surprisingly, two anti-Lys(Ac)-antibodies also bind a considerable number of peptides with other modifications (lanes 3 and 4) like lysine methylations and Ser/Thr phosphorylations. Additionally, we profiled an antibody directed against a specific histone mark (H4K16ac). Besides acetylated peptides the respective propionylated and butyrylated peptide derivatives are recognized, too (Figure 1, lane 6). Moreover, the antibody shows a broad subsite specificity resulting in strong binding to other acetylated histone marks which is further detailed in **Figure 2**. Several peptides with Kac modifications apart from the intended H4K16 site show high signals, especially if an arginine is succeeding the acetylated lysine residue.

Discussion & Conclusions

Histone Code Microarrays represent an efficient tool for reliable determination of substrate specificities of histone directed enzymes like arginine methyltransferase 5 (2), kinase Wee1 (3) or NAD^+-dependent histondeacetylase Sirtuin 3 (3) and their binding sites (4). Here we present an additional application of these microarrays: quality control of antibodies directed against post-translationally modified histones. A critical view on the quality of detection antibodies was taken recently in a Nature feature (5) pointing to problems with the specificity of detection antibodies, not only for epigenetic targets. So the validation of these antibodies is a critical and necessary step for sound scientific results. The knowledge on the specificity profile of any given antibody is indispensable for the valid interpretation of experimental results. The presented comprehensive Histone library allows a detailed view on the real targets for antibodies even including the single isoforms and an unprecedented set of modifications. Profiling results will allow the selection of the optimal reagent for your experiment, be it interaction studies, immunoprecipitation or ChIP experiments.

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The Author

Prof. Mike Schutkowski
mike.schutkowski@biochemtech.uni-halle.de
Chair of Enzymology
Martin-Luther University Halle-Wittenberg
Halle/Saale, Germany

Research Areas: Posttranslational protein modifications, binding specificity of protein domains, development of enzymatic assays, epigenetics

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