Tools of the trade

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Decoding the language of chromatin modifications with MARCS

Most eukaryotic DNA is stored in the nucleus as chromatin, which provides a framework for the regulation of genome functions. Chromatin consists of histones and DNA, both of which carry various chemical modifications that orchestrate DNA-templated processes by directing nuclear proteins to their target genomic loci. Many nucleosomes bear multiple interconnected modifications that modulate local genome accessibility and serve as binding platforms for proteins that can 'read' these modifications. However, despite progress in identifying 'readers' of individual modifications, how the nuclear proteome decodes complex modification landscapes remains unclear.

To address this question, we created a library of semi-synthetic dinucleosomes, incorporating modification signatures of promoter, enhancer and heterochromatin states. Using these dinucleosomes as baits in affinity purification experiments, we systematically examined the interactions between the human nuclear proteome and functionally distinct chromatin states by mass spectrometry[1]. We further developed computational tools for the analysis of this large-scale interaction proteomics dataset to quantitatively assess the direct effects of nucleosomal features on protein recruitment and exclusion. Using these tools, we identified networks of co-regulated factors, which connect chromatin modifications with downstream nuclear processes.

To make our findings readily accessible, we built the interactive web resource MARCS (Modification Atlas of Regulation by Chromatin States). MARCS offers a set of visualization tools to explore intricate chromatin regulatory circuits from either a protein-centred perspective or a modification-centred perspective (Fig. 1). Designed to distil complex nucleosome binding profiles into direct, actionable insights, MARCS can answer critical questions, including which modifications a particular protein recognizes, or what the bona fide readers of specific modifications are.

The MARCS 'Proteins' page facilitates exploration of the resource through the lens of a protein or nuclear complex of interest. Users can select from 1,915 identified nucleosome-interacting factors, including transcriptional activators and repressors, chromatin remodellers and modifiers, and DNA replication and repair factors. After selection of a protein or complex, the tool generates a heat map that depicts its binding responses to 55 chromatin-modification signatures representative of different chromatin states. Additionally, it produces a scatterplot that highlights the results of a feature effect analysis, which quantitatively estimates the direct impact of 15 distinct modification features on protein–nucleosome binding. When similar proteins are selected by the user, the tool extends this analysis to co-regulated factors that display similar binding profiles, automatically visualizing them as networks in a 'Neighbourhood' panel. This functionality allows users to determine whether proteins of interest engage with nucleosomes individually or as part of a complex, which enables the prediction of novel protein–protein interactions. Notably, the algorithm also identifies proteins with symmetrically opposite binding profiles, thereby expanding the selection to include factors with contrasting modification-driven responses. The complete set of co-regulated protein clusters is provided in the 'Network' page.

The 'Chromatin Feature Effects' page facilitates browsing of the resource by nucleosomal feature, which includes 13 lysine methyl and acetyl modifications on histones H3 and H4, DNA methylation and the histone variant H2A.Z. After selection of a specific feature, the tool generates a volcano plot that highlights the responding proteins. Importantly, unlike genome-wide mapping-based tools that can only infer correlations between modifications and chromatin-associated proteins, our resource identifies proteins whose binding is directly modulated by a particular feature. For example, although histone H3 lysine 27 acetylation (H3K27ac) is a hallmark of active enhancers, it only marginally stimulates the binding of few factors, such as the SRCAP complex, but does not affect the binding of key enhancer-associated proteins, such as BRD4 or the BAF (SWI/SNF) complex. In line with recent findings[2], this observations suggests that H3K27ac does not have a direct role in mammalian transcriptional regulation.

Additionally, MARCS offers the 'Pull-downs' tool, which allows users to browse the resource by specific chromatin-modification signatures, highlighting the

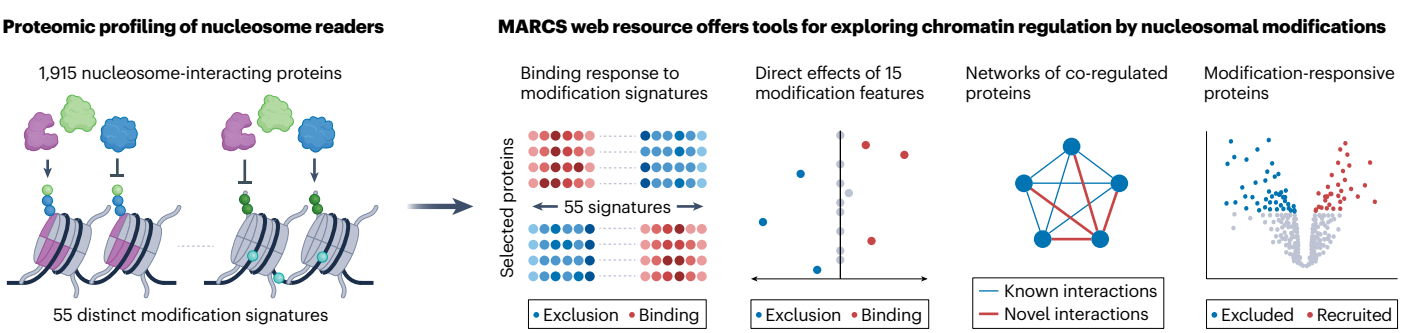

Fig. 1 | The MARCS web resource. MARCS unifies a modification-centric view with a protein-centric view of chromatin to facilitate the exploration of chromatin regulation.

responding proteins. The MARCS toolbox is designed to bridge the gap between chromatin modifications and their functions, serving as a platform for both hypothesis generation and validation. We encourage researchers to delve deeply into this unique resource to uncover the numerous insights it offers.

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Competing interests
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References
1. Lukauskas, S. et al. Decoding chromatin states by proteomic profiling of nucleosome readers. *Nature* **627**, 671–679 (2024).
2. Sankar, A. et al. Histone editing elucidates the functional roles of H3K27 methylation and acetylation in mammals. *Nat. Genet.* **54**, 754–760 (2022).