PROTEOMICS

Identifying the chromatin-associated proteome

ChromID identifies proteins associated with various chromatin modifications.

Chromatin is marked by a variety of chemical modifications on histones — such as acetylation, methylation and phosphorylation — that impart unique regulatory control of genomic expression and function. These chemical modifications are identified by specific regulatory proteins bearing ‘reader domains’. However, neither the protein composition nor the details of the interaction between the various chromatin-associated proteins and chromatin are fully understood. Baubec and colleagues at the University of Zurich have recently developed a systematic approach to characterize the proteome associated with specific chromatin modifications.

First, the researchers characterized the specificity and affinity of known reader domains to specific chromatin modifications in mouse embryonic stem cells. Using green fluorescent protein–tagged reader domains, the authors performed live-cell imaging to visualize their nuclear localization and identified their genomic binding by ChIP-seq; pull-down assays were used to determine specific interactions with chromatin modifications. The characterized reader domains were then used to engineer synthetic proteins that the authors call engineered chromatin readers (eCRs), which they developed to be selective for DNA methylation and histone trimethylation. eCRs were built by combining individual domains for combinatorial binding to multiple histone marks or were fused to the bacterial enzyme BASU, a promiscuous biotin ligase that rapidly biotinylates proteins in an ~10-nm radius around the site of localization (proximity biotinylation). In this case, the site of localization is guided by the specificity of the associated eCR. This eCR–biotin ligase construct is the basis of the method that the authors term ChromID, which enables identification and quantification of the proteome surrounding specific histone modifications.

Using ChromID, the researchers detected proteins directly and indirectly associated with the methylated histone H3 residues H3K4me3, H3K27me3 and H3K9me3 in

mouse embryonic stem cells. More than 500 proteins were identified, 180 of which were found to be enriched across all datasets. By using a bivalent reader, the authors also identified the proteins bound at bivalently modified promoters marked by H3K4me3 and H3K27me3. Specific proteins were identified to be activating or repressing for chromatin regulation. “While many of the reported proteins that we have identified with different chromatin marks were known already, our characterization enabled [us] to identify several novel factors that we did not expect to find. It will be interesting to find out more about the role of these factors in the respective chromatin context,” says Baubec regarding their results.

ChromID is unique in providing a simple modular approach based on naturally occurring elements that can be used in multiple combinations for systematic characterization of the proteome. It also overcomes the challenges associated with established techniques in the field that typically require cross-linking of proteins to the chromatin or access to the underlying DNA — often disrupting the chromatin. ChromID has paved the way for making in vivo studies a reality. However, the approach has several limitations. Natural reader domains tend to have low affinities; design of high-affinity constructs therefore requires multiple rounds of design and testing. Additionally, biotinylation experiments in a crowded space, such as the nucleus, lead to very noisy results requiring many adjustments and optimization steps. Baubec’s group is working on improving the signal-to-noise ratio, as well as further optimizing the method to work with lower sample amounts and applying it to in vivo models.

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