ABSTRACT

CHROMATIN AND TRANSCRIPTIONAL REGULATION IN MOUSE MACROPHAGES

By

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Eukaryotic genomes must be extensively packaged into a DNA-protein complex called chromatin due to their large sizes and the spatial restrictions of the nucleus. Nucleosomes, the basic repeating unit of this complex, have long been viewed as a barrier to basic cellular processes including transcription, and recent studies suggest that chromatin architecture plays a critical role in the regulation of gene expression. We have used primary bone marrow-derived macrophages (BMDMs) as a model to investigate chromatin changes associated with inducible and cell-type specific gene expression in response to bacterial lipopolysaccharide (LPS). Macrophages are specialized cells of the innate immune system that arise during differentiation from multipotent hematopoietic stem and progenitor cells (HSPCs) through the coordinated action of lineage-specific transcription factors (TFs). These cells have unique functions in response to foreign threat, and previous genome-wide studies have identified macrophage-specific distal enhancers that play a key role in the pro-inflammatory response to LPS. Using a quantitative nucleosome occupancy assay, we have shown that nucleosomes are stably evicted from these enhancers under inducing conditions in BMDMs, and this depletion correlates with signal-induced TF binding and increased gene expression. Using a knockdown approach targeting BAF/PBAF chromatin remodeling complexes, we have shown that nucleosome remodelers are recruited to regulatory elements early during differentiation by lineage-specific TFs, and that disruption of this process results in increased nucleosome occupancy at these elements and prevents nucleosome eviction and gene induction in response to LPS. In order to more precisely determine how and when enhancers might be rendered accessible during differentiation, we further investigated chromatin structure in HSPCs. This led to the surprising finding that nucleosome occupancy may be universally low in these cells.

We are now using a genome-wide extension of the quantitative nucleosome occupancy (GNO-seq, Global Nucleosome Occupancy-sequencing) to analyze changes in nucleosome occupancy associated with macrophage differentiation from HSPCs genome-wide. This research will provide crucial insights into the regulation of inducible gene expression, the role of remodelers in maintaining chromatin accessibility, and may demonstrate global differences in chromatin between cell types.