ABSTRACT

DUAL ROLE OF PU.1 IN ENHANCER PRIMING IN MACROPHAGES

By

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All multicellular organisms arise from a single-celled zygote by the precise execution of a gene expression program which ensures appropriate cell identity. This process is particularly challenging in eukaryotic cells since eukaryotic DNA is packaged by architectural proteins called histones into chromatin, which might act as a barrier to the transcriptional machinery. Macrophages are cells of the immune system which undergo rapid, large scale changes in gene expression in response to bacterial or viral challenge. This makes macrophages an excellent model for studying cell-type specific as well as inducible gene expression. Studies at the genome-wide level have shown that distal regulatory elements like enhancers play an essential role in determining the macrophage inducible response to microbial challenge. Further, lineage-specific transcription factors like PU.1 and C/EBPβ are known to bind inducible enhancers prior to gene induction in resting macrophages. Earlier studies using genome-wide approaches indicate that PU.1 is able to interact with chromatin, thus functioning as a 'pioneer factor' in macrophages. However, not much is known about the mechanism by which PU.1 keeps enhancers accessible prior to gene induction in resting macrophages. Using bone-marrow derived primary mouse macrophage cells as well as PU.1 deficient cell lines, my work highlights the changes in chromatin associated with PU.1 binding during macrophage differentiation as well as in response to bacterial infection. Using a quantitative nucleosome occupancy assay, we reported that PU.1 binding correlates with low nucleosome occupancy at an inducible enhancer in resting macrophages. Further upon induction with an appropriate stimulus, nucleosomes are stably evicted from the distal enhancer and the corresponding gene can be induced. More importantly, my results suggest that lack of PU.1 binding renders regulatory regions (enhancers and promoters) of inducible genes susceptible to heterochromatin formation and silencing by Polycomb repressive complex 2 (PRC2) in

differentiated macrophages. PRC2-mediated silencing is also associated with an increase in nucleosome occupancy at the target regions and the corresponding genes cannot be induced. Results obtained from this research will provide important insights into the role of lineage-specific transcription factors at regulatory elements both during normal development and disease.