Transcription factor sensitivity to DNA methylation

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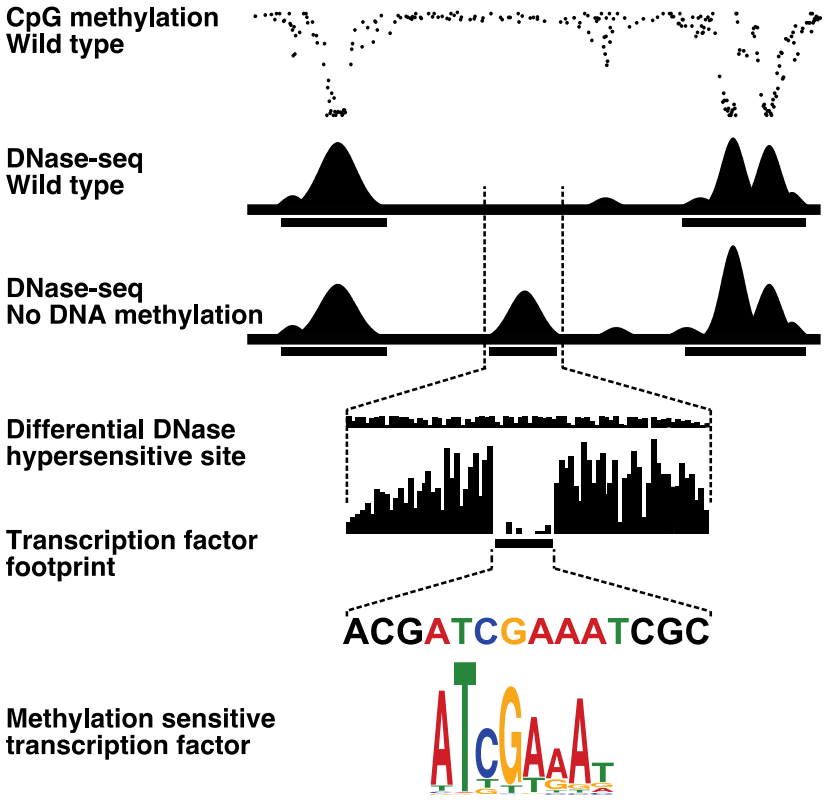
Active regulatory regions are occupied by transcription factors (TFs) and display a defined chromatin state. While the relevance of chromatin in gene regulation is undisputed, it is controversial whether a chromatin state is a consequence of or prerequisite for TF binding. Since TFs differ in their sensitivity to chromatin (e.g. pioneer TFs), the relationship between chromatin and TF binding is factor-specific and context-dependent. For example so called “pioneer TFs” are thought to trigger changes in chromatin as they bind non-accessible chromatin and recruit remodeling complexes that can displace or remove nucleosomes from regulatory regions to facilitate binding of context-specific TFs. Other factors appear to be sensitive to nucleosome positioning, histone or DNA modification. Besides a few well-studied cases, we have no comprehensive knowledge about the actual sensitivity of TFs to chromatin states.

Several chromatin pathways exist that have been linked to gene repression in higher eukaryotes including covalent histone modifications and DNA methylation at cytosines within CpG dinucleotides. Even though these pathways are essential for proper development, it is unclear how they affect TF binding. Moreover it can be misleading to generalize from individual loci. For example, extensive studies have shown that CTCF binding at the H19-Igf2 imprinted locus is sensitive to DNA methylation. However our laboratory has shown that CTCF binding is not affected by DNA methylation outside of CpG rich regions in stem cells that lack DNA methylation.

This study aims at measuring the effect of DNA methylation on the genomic binding landscape of TFs (see figure). We use the formation of

DNase I hypersensitive sites (DHS) as a comprehensive indicator of TF binding and monitor genome-wide changes in DHS in presence or absence of DNA methylation. This reveals that removal of DNA methylation in mouse embryonic stem cells does not globally perturb the DNase I landscape and in turn argues that binding of most TFs expressed in stem cells is not limited by DNA methylation. Importantly however we also observe that defined TFs that recognize sequence motifs containing CpGs, reveal sensitivity and appear to compete with DNA methylation for binding.



We validate this prediction for a particular factor by several means. First ChIP-seq shows that upon loss of DNA methylation it indeed binds a substantial number of new sites that were previously methylated in wildtype. Moreover, we show that restoring DNA methylation initiates remethylation at these sites and outcompetes its binding. Finally we observe that the binding sites of TFs sensitive to DNA methylation are already occupied by TFs insensitive to DNA methylation. Indeed we show that removal of other TF motifs in *cis* or TFs in *trans* causes local methylation and loss of binding of a

methylation sensitive factor. Therefore, binding of DNA methylation insensitive TFs enables sensitive TF binding sites to become unmethylated and accessible. This work reinforces the principles of cooperative binding of transcription factors and shed light on their interplay with DNA methylation.