Correlation between Histone Modifications and 5-hydroxymethylation in Human Brain

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Abstract

Histone tails that are exposed at the nucleosomal surface are targets for covalent modifications such as acetylation, methylation, and phosphorylation. These post-translational modifications play crucial roles in the regulation of chromatin structure and maintenance of genomic stability. DNA methylation of CpG dinucleotides is another epigenetic event involved in gene regulation. Recent studies demonstrated that 5-methylcytosine (5-mC) can be further modified to 5-hydroxymethylcytosine (5-hmC) by the ten-eleven translocation (Tet) family proteins. The balance between 5-mC and 5-hmC in the genome is a critical step for regulating gene expression to maintain cellular functions. 5-hmC is particularly enriched in the brain when compared to other tissues. However, the biological mechanisms linking histone modifications and 5-hmC in human brain and other tissues have not yet been established. Reduced Representation Hydroxymethylation Profiling (RRHP) is a method that combines whole-genome library preparation with selective adapterization and Next-Gen sequencing. Using RRHP, a new technique that map 5-hmC at single-nucleotide resolution in a strand-specific fashion, we analyzed the 5-hmC profiles of human male cerebellum. The correlations between 5-hmC and histone modifications are assessed using functional annotation analysis. We observed that 5-hmC enriched regions overlapped with H3K4me3 (a euchromatin mark)-associated regions, accounting for more than 50% of the overlap, whereas, only 25% of 5-hmC sites overlapped with H3K27me3 (a transcription repressive mark)-associated regions. Additionally, we also observed 12% of 5-hmC overlapped with bivalent domains which consisting both H3K4me3 and H3K27me3 marks. Taken together, our newly developed technique, RRHP provides new insight into the relationship between 5-hmC and histone modifications, and will be a powerful tool for future studies on the diverse regulatory roles associated with 5-hmC and histone modifications.

Workflow

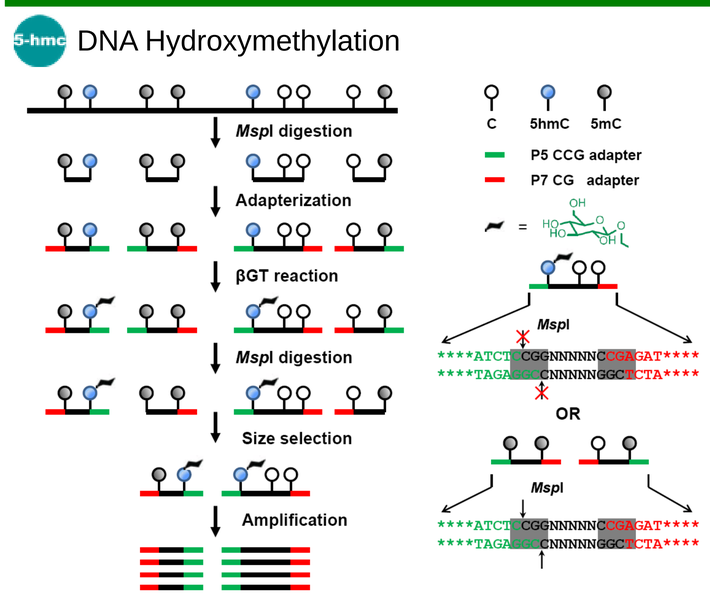
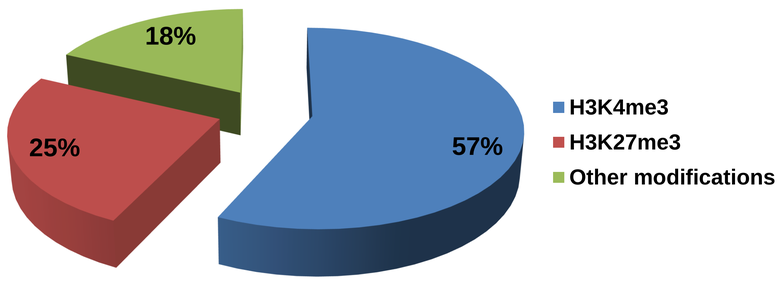


Figure 1. Schematic overview of the Reduced Representation Hydroxymethylation Profiling (RRHP) system. The assay exploits β-glucosyltransferase (β-GT) to selectively label 5-hmC positions at adapter junctions, thus preventing digestion of the adapter away from the fragment. Fragments lacking 5-hmC at the junction will not be labeled and the adapter can be digested away. Only fragments with intact adapters on both sides will be amplified for hybridization and sequencing.

Results

A Correlation between 5-hmC and histone modifications



B Venn-diagram of H3K4me3- and H3K27me3-enriched genes

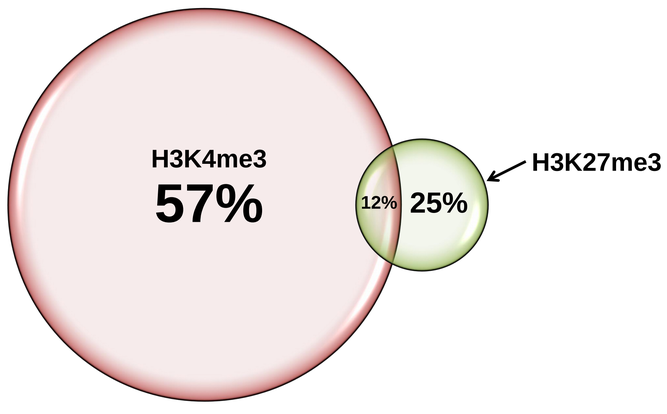


Figure 2. 5-hmC is enriched in both actively transcribed and repressed genes. Correlations between 5-hmC and histone modifications are assessed using functional annotation analysis. ***(A)*** Pie chart representation of genomic regions with enriched 5-hmC and H3K4me3 (a euchromatin mark) or H3K27me3 (a transcription repressive mark) or other histone modifications. ***(B)*** Scaled Venn diagram represents distribution of 5-hmC sites in H3K4me3- or H3K27me3-enriched regions. Intersection between the two marks represents 5-hmC mapping to bivalent regions.

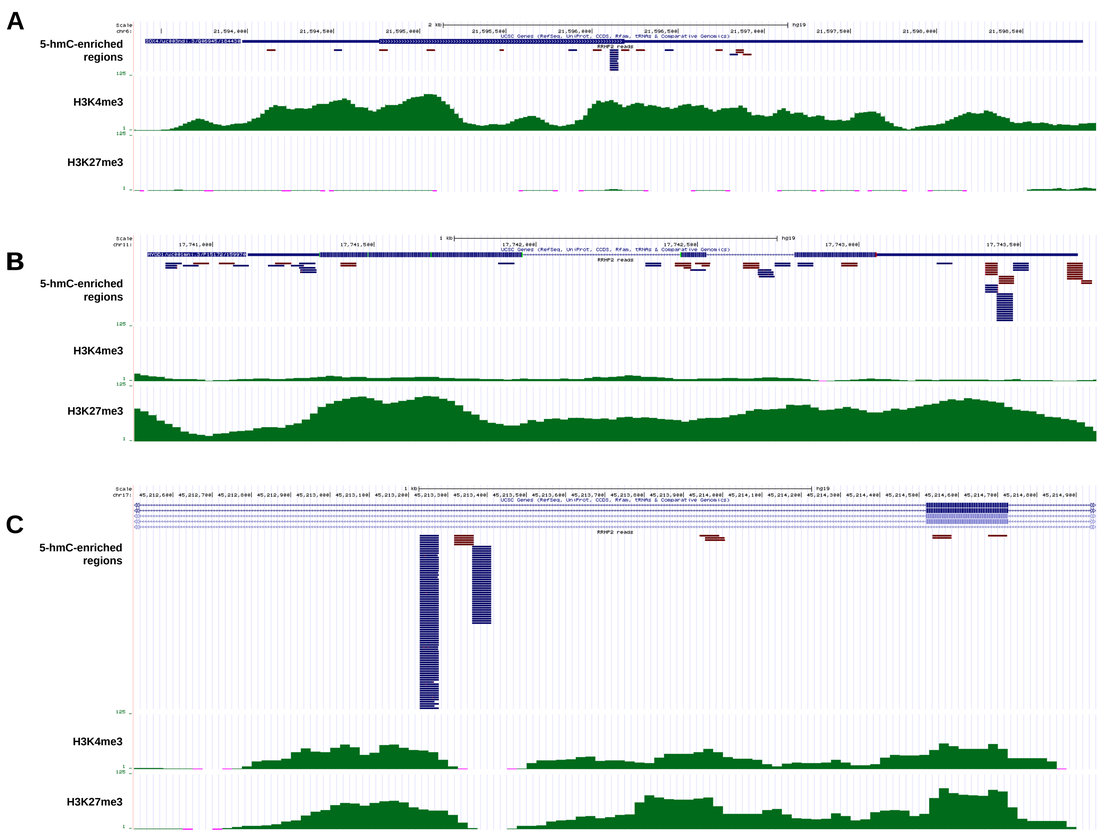


Figure 3. 5-hmC is enriched in both actively transcribed and repressed genes. Shown are profiles of ***(A)*** H3K4me3, ***(B)*** H3K27me3, and ***(C)*** bivalent regions occupancy at 5-hmC targets.

Conclusion

Our RRHP offers a strong, positive-display output for stringent identification and mapping of 5-hmC across the genome. By correlating genome-wide distributions of 5-hmC with histone modifications, 5-hmC displayed relatively strong correlations with H3K4me3 when compared to H3K27me3. This suggests potential role(s) for 5-hmC in the regulation of gene expression. Taken together, RRHP provides new insight into the dynamic relationship on both DNA and histone modifications, and RRHP will be a powerful tool for exploration of a novel aspect of the intricate epigenetic regulations.

References

1. Pan G, *et al.* (2007) Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. **Cell Stem Cell** 1(3):299-312.
2. Bernstein BE, *et al.* (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. **Cell** 125(2):315-26.

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