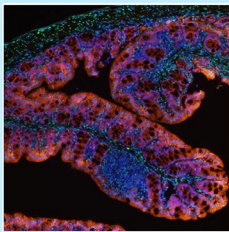




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Mouse Colon
(blue: DAPI, green: EPCAM, and red: 5hmC)

Mapping 5-Hydroxymethylcytosine in the Intestinal Epithelium and Assessing Its Impact on Gene Expression

Chromatin regulation plays a role in establishing and maintaining cell identity, and is generally highly dynamic between stem and differentiated cells. The intestinal epithelium is a monolayer of cells that is replaced every 3-5 days, and is thus a rapidly self-renewing and differentiating tissue. It is not currently understood how the integrity of the intestinal stem cell (ISC) genome is maintained or how the identity of a differentiated cell is dictated. Vital to chromatin regulation and gene expression are epigenetic modifications, such as methylation. The addition of methyl groups to cytosines silences genes, while demethylation allows gene expression. Demethylation pathways produce the epigenetic marker 5-hydroxymethylcytosine (5hmC) as an intermediate. The conversion of 5-methylcytosine (5mC) to 5hmC is accomplished by ten-eleven translocation (TET) enzymes, which require co-factors, such as the micronutrient alpha-ketoglutarate (a key intermediate in Krebs cycle). Assessing the global 5hmC abundance among distinct cellular populations will elucidate this epigenetic mark's role in determination of cell fate. To investigate this aim, murine intestinal organoid cultures were exposed to varying dosages of 2-oxoglutarate, a cell permeable form of alpha-ketoglutarate, in order to modulate TET activity and thus 5hmC abundance. Through qPCR, immunohistochemistry, and dot blot analysis of 5hmC abundance, the impact of 5hmC on gene expression was assessed. We observed increased 5hmC over coding regions of genes associated with absorptive enterocytes, and decreased 5hmC enrichment over coding regions of genes associated with secretory cell types. Understanding the differences in 5hmC across cellular populations will provide insight into the role of epigenetic modifications in regulating gene expression. Furthermore, the impact of micronutrients on the genome emphasizes the significance of cellular environment for cellular identity.