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Grant Title: A novel role of chromatin glycosylation in the epigenetic control of ribosome biogenesis

This project aimed at uncovering the underlying epigenetic mechanism by which intracellular glycosylation, specifically O-GlcNAc modification, controls ribosome biogenesis. This project builds on previous observations of a downregulation of ribosome biogenesis upon perturbation of O-GlcNAc homeostasis. Here, we sought to determine whether: 1) OGT and O-GlcNAc modification may directly activate rDNA transcription via glycosylation of rDNA-bound proteins, and 2) whether the hypothetical regulatory role of OGT in rDNA transcription implicates its binding partners TET1 and TET2.

We developed a CRISPR-based epigenetic editing system with high spatial genomic resolution to perturb O-GlcNAc levels specifically at rDNA chromatin. This system utilizes deactivated Cas9 (dCas9) fused to O-GlcNAc-modifying enzymes (either OGT or a bacterial OGA homolog BtGH84) and specific guide RNAs targeting the promoter of the 45S rDNA. We quantified nascent transcription of the rDNA by measuring the unprocessed rRNA using droplet digital PCR. Additionally, we employed microscopy techniques to analyze OGT localization within nuclear compartments, focusing on the nucleolus, the site of rDNA transcription.

Our investigation has yielded unexpected findings with regards to our initial hypotheses. First, we established an epigenetic editing system in mouse embryonic stem cells to perturb OGlcNAc levels either positively (using OGT) or negatively (using Btgh84) specifically at rDNA chromatin. Surprisingly, neither decreasing nor increasing O-GlcNAc levels at the rDNA promoter had a measurable influence on nascent transcription of rRNA. Hense, the mechanism by which O-GlcNAc sustains ribosome production is likely indirect. Microscopy experiments revealed that OGT is depleted in the nucleolus as compared to the nucleoplasm, providing a potential explanation for these results. Altogether, these findings suggest that the relationship between O-GlcNAc modification and ribosom biogenesis operates through indirect mechanisms that require further investigation.