Principal Investigator: David Vocadlo
Grant Title: In vivo genome wide dynamics of O-GlcNAcylated chromatin-associated proteins

Abstract: Advances in DNA sequencing are enabling new data-intensive experimental modalities for studying chromatin. One emerging area is to understand the time-dependent changes that occur to the genome using a series of ChIP-seq experiments performed at different times. Using new chemistries in combination with sequencing to study chromatin has permitted TC studies to monitor proteins, DNA modifications, and DNA lesions. Although chemoselective labeling strategies for many protein modifications are rapidly advancing, few such methods have been shown to enable mapping protein modifications to chromatin. Further, despite the crucial roles played by protein modifications in regulating gene expression, no methods have been developed to enable monitoring time-resolved turnover of protein modifications on chromatin-associated proteins. To address this gap we have developed a combined chemical genetic method to examine time-resolved changes to post-translational modifications of chromatin-associated proteins.

Here we use DNA precipitation methods to enable monitoring time-dependent turnover of *O*-GlcNAc modified proteins associated with chromatin. Using an antibody-free chemical reporter strategy, we performed time course metabolic feeding experiments with wild-type *Drosophila* larvae alongside larvae lacking *O*-GlcNAc hydrolase (OGA), which are accordingly unable to remove *O*-GlcNAc (Figure 1). Analysis of resulting next-generation DNA sequencing data enabled mapping of *O*-GlcNAc to the genome and revealed that *O*-GlcNAc on chromatin-associated proteins at most genomic loci are processed with a half-life in hours. Notably, loss of OGA only increases the half-life by ~3-fold. Interestingly, a small set of genomic loci are particularly sensitive to loss of OGA. In addition to these observations and new strategies to permit monitoring turnover of *O*-GlcNAc on chromatin, we also detail methods for coded blinding of samples alongside new normalization strategies to enable time-resolved genome-wide analyses using chemical genetic methods. Given the essential roles played by nutrient-regulated *O*-GlcNAc in controlling gene expression, coupled with the beneficial effects of modulating OGA activity in various disease models, methods to understand its dynamic nature on the genome should prove useful. Here, using wild-type (WT) and OGA knockout (OGA-null) flies in combination with TC *O*-GlcNAc-seq, we determine relative rates of turnover of *O*-GlcNAc on the genome and show that different genomic loci vary in their behavior. We envision this method and normalization strategies will be applicable to diverse protein and nucleic acid modifications.

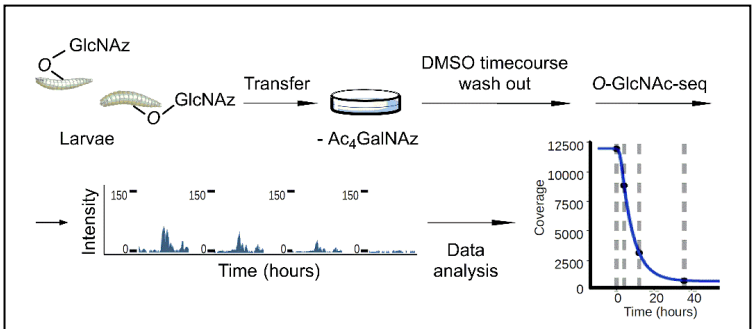

Figure 1: Timed feeding of metabolic precursors, followed by click chemistry and next-generation sequencing, enables monitoring time-resolved changes in post-translational modifications in a genome-wide manner.

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