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Regulatory Function of the Groucho Corepressor in *Drosophila* Development

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by

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**ABSTRACT OF THE DISSERTATION**

Regulatory Function of the Groucho Corepressor in *Drosophila* Development

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Animal developmental patterning is a complex and intricate process, requiring the interpretation of multiple temporally and spatially regulated signals to define the transcriptional profile of each cell. In *Drosophila*, Groucho (Gro), a transcriptional corepressor, participates in these processes through long-range silencing of distant enhancers. Lacking any innate DNA-binding activity, Gro is targeted to these elements through interaction with multiple repressors.

Using a combination of ChIP-seq and RNA-seq techniques, I sought to characterize Groucho activity at multiple stages spanning the initial nine hours of embryonic development, and thereby gain insight into both the mechanisms and extent of Groucho-mediated repression. Groucho is recruited to thousands of sites at each stage of development. Most Gro peaks are < 1kb in width, consistent with recruitment of one or a small number of Gro complexes to a regulatory element, indicating that spreading of Gro along stretches of chromatin is not a common feature of repression, as previously thought. Gro binding is frequently observed as clusters of discrete peaks, which supports a model in which Gro self-association facilitates crosslinking of non-contiguous regions of chromatin. In some cases, Gro occupancy is observed at transcriptional start sites multiple kilobases from known Gro-binding silencing elements, suggesting that interactions with these sites via crosslinking and looping of chromatin is one method of Gro-mediated repression.

Both Gro gain- and loss-of-function embryos exhibit extensive perturbations in gene expression from the onset of zygotic transcription. Integration of these differentially expressed genes with ChIP-seq-derived Gro occupancy data facilitated the identification of Gro targets within each developmental stage, including known Gro-regulated genes and novel targets. Gro target genes are enriched for transcription factors involved in multiple developmental processes, as well as regulators of multiple signaling pathways. The activity of Gro in regulating both the inputs and outputs of signaling pathways suggests that Gro is utilized to regulate the cellular response to signaling on multiple levels by facilitating crosstalk between pathways.

Gro binds to many genes that it does not repress as exemplified by genes that are regulated by Dorsal, a bifunctional transcription factor that activates some targets and represses others. Gro is essential for repression, but dispensable for activation by Dorsal. However, data presented in this thesis suggest that Dorsal recruits Gro to both the repressed and the activated targets. This shows that Gro recruitment is not sufficient for repression.

I used a technique to isolate and sequence the nascent transcripts of actively transcribed genes to obtain an accurate profile of the transcriptional activity of the *Drosophila* embryo at multiple stages of development. I find that nascent transcripts of Gro target genes are significantly enriched for promoter-proximal read density, indicating that Gro targets are enriched for stalled PolII. Coupled with the observation that Gro often binds overlapping or shortly downstream of start sites, we propose that Gro may repress genes by favoring the stalling of PolII.

Finally, I have contributed to a study characterizing the Gro interactome. This study showed that Gro interacts with U1 snRNP. I compared the effect of knocking down a U1 snRNP subunit with that of knocking down Gro on the S2 cell transcription profile. The results suggest that Gro-mediated repression of some targets may require U1 snRNP.