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The Central Region of the Drosophila Co-Repressor Groucho as a Regulatory Hub

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Running title: *Groucho/Spliceosome Interactions*

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Background: The co-repressor Groucho has an essential, but disordered, central region.
Results: We identified over 160 central region-binding proteins, many of which, including components of the spliceosome, modulate Groucho-mediated repression.
Conclusion: Groucho regulates transcription by multiple mechanisms and may link the transcriptional and splicing machineries.
Significance: Its central region may serve as the hub of a regulatory network.

ABSTRACT

Groucho (Gro) is a Drosophila co-repressor that regulates the expression of a large number of genes, many of which are involved in developmental control. Previous studies have shown that its central region is essential for function, even though its three domains are poorly conserved and intrinsically disordered. Using these disordered domains as affinity reagents, we have now identified multiple embryonic Gro-interacting proteins. The interactors include protein complexes involved in chromosome organization, mRNA processing, and signaling. Further investigation of the interacting proteins using a reporter assay showed that many of them modulate Gro-mediated repression either positively or negatively. The positive regulators include components of the spliceosomal subcomplex U1 small nuclear ribonucleoprotein (U1 snRNP). A co-immunoprecipitation experiment confirms this finding and suggests that a sizable fraction of nuclear U1 snRNP is associated with Gro. The use of RNA-seq to analyze the gene expression profile of cells subjected to knockdown of Gro or snRNP-U1-C (a component of U1 snRNP) showed a significant overlap between genes regulated by these two factors. Furthermore, comparison of our RNA-seq data to Gro and Pol II ChIP data led to a number of insights including the finding that Gro-repressed genes are enriched for promoter proximal Pol II. We conclude that the Gro central domains mediate multiple interactions required for repression thus functioning as a regulatory hub. Furthermore, interactions with the spliceosome may contribute to repression by Gro.

Groucho (Gro) is a conserved metazoan co-repressor that may be particularly critical for