long-range repression, whereby repressors are able to establish large transcriptionally silent domains that can spread over many thousands of basepairs (1-3). Gro is essential in many developmental processes, including sex determination, neurogenesis, and pattern formation in Drosophila, as well as myogenesis and hematopoiesis in vertebrates (2,4,5). Gro also has roles in multiple signal transduction pathways, including the Ras and Notch pathways (6-8). Furthermore, increased Gro activity correlates with the appearance of certain forms of cancer, such as lung cancer (9,10). Thus, understanding the mechanism of Gro-mediated repression should contribute to our understanding of long-range repression and its role in development, signaling, and disease.

Sequence comparison of Gro family proteins reveals five domains (2,10). The C-terminal WD-repeat domain forms a β-propeller that interacts with the WRPW and eh1 motifs found in many Gro-dependent DNA-binding repressors (11). The N-terminal Q domain folds into a coiled-coil structure that forms tetramers and perhaps higher order oligomers, and this self-association is required for robust repression (12-15). The central GP, CcN, and SP domains are believed to have essential functions even though their primary sequences are not well conserved. The GP domain interacts with the histone deacetylase Rpd3/HDAC1 (16,17). Histone deacetylation is broadly associated with gene silencing, and treatment of flies with histone deacetylase inhibitors attenuates Gro-mediated repression (18). In addition, the GP domain is essential for nuclear localization, since deletion of this domain prevents Gro nuclear uptake (19). The SP domain regulates Gro function negatively, as its deletion leads to promiscuous repression and developmental defects (19). Phosphorylation of the SP domain by Ras/MAPK signaling was shown to attenuate repression, providing a mechanism for regulating repression in response to environmental cues (20). Finally, the CcN domain is also targeted for phosphorylation by protein kinases and is required for repression by Gro (19,21).

Sequence analysis of the Gro central domains strongly suggests that they are intrinsically disordered (19). Intrinsically disordered regions in proteins lack rigid three-dimensional structures under native conditions and can serve as hubs of large regulatory networks by mediating a wide array of highly specific protein interactions (22,23). Increasing evidence suggests that intrinsically disordered domains have critical functions in transcriptional regulation (24,25).

In this study, we set out to illuminate the mechanisms of Gro mediated repression by identifying proteins that interact with the N-terminal Q domain and the three central domains. A proteomic screen revealed over 160 interacting proteins, many of which are components of protein complexes in a variety of functional categories such as chromatin remodeling and RNA processing. Perhaps most notably, the interactors included multiple components of the spliceosome, and a co-immunoprecipitation experiment suggests that a sizable fraction of U1 snRNP (a subcomplex of the spliceosome) is associated with Gro in embryonic nuclei.

As a means of systematically validating the functional significance of these interactions, we carried out a novel reporter assay employing three different luciferase reporters that could be monitored simultaneously. These assays showed that many of the interacting proteins, including the protein components of U1 snRNP, are required for optimal Gro mediated repression. Lastly, we compared the effects on gene expression profile of Gro and U1 snRNP knockdown, finding a significant overlap in the regulated genes. Our results indicate that the central domains of Gro mediate multiple interactions required for repression, and reveal a possible mechanism of Gro mediated repression through an interaction with the spliceosome complex or subcomplexes. This reinforces previous studies suggesting that the spliceosome has roles in transcriptional