shown to repress transcription of EWS/FLI-transactivated genes (30). Since our data indicated that snRNP-U1-C may also modulate Gro function, we examined the genome-wide role of snRNP-U1-C in Gro mediated repression. Using RNA-seq, we compared the effects of snRNP-U1-C knockdown to that of Gro knockdown on the gene expression profile in S2 cells. Cells were treated with Gro or snRNP-U1-C dsRNA for four days, leading to four-fold or greater knockdown of the Gro and snRNP-U1-C mRNA (Figure 4A). The transcriptomes in wild-type and Gro knockdown S2 cells were quantitatively similar to those published previously (49,50) (Figure 4B, C). We note that the genes differentially expressed in the snRNP-U1-C knockdown are enriched for genes containing introns as would be expected given the role of U1 snRNP in splicing. However, this set of genes also contains a number of intron-less genes consistent with the idea that snRNP-U1-C has roles in gene regulation apart from its role in splicing (Figure 4D). We note that changes in the expression of an intron-less gene could also reflect a requirement for the product of an intron-containing gene in the expression of the intron-less gene.

98 genes were differentially expressed in both Gro and snRNP-U1-C knockdown cells (Figure 4E), of which 36 were upregulated in either case. These coordinately upregulated targets included genes in various signaling pathways, such as the Wnt, Notch, and Toll pathways (Table 6). Comparison with publically available ChIP-seq data on histone modification and transcription factor binding revealed that these coordinately regulated genes were most enriched for histone H3K36 methylation and the H3K36 methyltransferase ASH1 (Figure 4F).

To determine if the regulatory effects of knocking down Gro are likely to be direct, we compared our RNA-seq data from Gro knockdown S2 cells to available S2 cell Gro ChIP data (49). Gro appears to bind many genes that it does not repress (Figure 5A). This is consistent with observations made with numerous regulatory factors (51,52) and suggests that binding, while required, is not sufficient for regulation. We observe an enrichment of Suppressor of Hairless (Su(H)) and Brinker (Brk) binding motifs within Gro ChIP-seq peaks in the differentially expressed genes but not in the non-differentially expressed genes (Figure 5B). Comparison of our RNA-seq data from Gro knockdown cells to available Pol II ChIP-chip data (53) also reveals an enrichment in Pol II pausing near the transcriptional start site in genes that are up-regulated upon Gro knockdown (i.e., genes that are repressed by Gro; Figure 6).

DISCUSSION

Previous studies showed that the disordered Gro central domains are essential for properly regulated transcriptional repression (2,19). To shed light on the mechanism by which these domains function, we used them as affinity reagents to purify interacting proteins in Drosophila embryo nuclear extracts, which were then identified by MuDPIT. We identified over 160 interacting polypeptides, many of which associate with one another in a variety of multi-protein complexes. Several of these interacting proteins (e.g., the core histones, CKII) were previously characterized as Gro interactors thus partially validating the screen. In addition, we validated the interaction between Gro and U1 snRNP by demonstrating the presence of U1 snRNA in an anti-Gro immunoprecipitate of embryonic nuclear extracts.

As a means of systematically validating interactions, we employed a functional assay in Drosophila cells, in which 157 of the interactors were each knocked down by RNAi to determine their requirement for Gal4-Gro-mediated repression of a luciferase reporter. In this way, we obtained evidence that 44 of the interactors have functional roles in Gro mediated repression. 28 of these are required for repression while 16 of them antagonize repression. The number 44 is probably an underestimate of the true number of functional