interactors due to the artificiality of the reporter assay. For example, because we artificially recruit Gro to the reporter by tethering it to the Gal4 DNA binding domain, any interactions that work to help recruit Gro to the template will not be required. In addition, the reporters are introduced by transient transfection, and certain chromatin structures or modifications that contribute to Gro-mediated repression may not be reproduced in this context.

Gro-interactors include chromatin remodelers, protein kinases, and protein complexes involved in RNA processing–Gro-mediated repression may be associated with changes in chromatin structure including histone deacetylation and possibly increased nucleosome density (3,18,54). Consistent with this possibility, our proteomic screen identified a number of histone modifiers and ATP-dependent chromatin remodelers, including subunits of the ACF chromatin remodeling complexes (Acf1 and Iswi), the histone chaperone NAP1, and the histone kinases JIL-1 and Ball. Consistent with the idea that chromatin remodelers may be required for Gro-mediated repression by catalyzing changes in nucleosome density or higher order chromatin structure, our reporter assay showed that Acf1 is required for optimal repression by Gro.

CKII is a heterotetrameric complex consisting of two copies of a catalytic subunit (CKIIα) and two copies of a regulatory subunit (CKIIβ) (55,56). A previous study showed that CKII phosphorylates Gro at multiple sites including serines 239 and 253 to promote repression (21). We identified both the α and β subunits of CKII and the CKII negative regulator Nopp140 in our proteomic screen; but our findings are inconsistent with the view that CKII is a positive regulator of Gro and that Nopp140 acts by inhibiting CKII. This is because our reporter assays show that CKIIα, CKIIβ, and Nopp140 are all negative regulators of Gro. However, our results are consistent with other findings showing that Gro phosphorylation can block repression (2). Furthermore, the effect we observe due to Nopp140 knockdown could reflect the role of this factor in processes other than CKII regulation (57).

In addition to several expected protein complexes, we have also isolated many novel Gro interacting proteins, one of which is the RNA helicase Rm62 (also known as p68). Rm62 is a DEAD box RNA helicase that has multiple functions including roles in RNA processing, RNAi, and transcriptional regulation (58). Previous studies have shown a dual role for Rm62 in transcriptional regulation – its interaction with coactivator CBP/p300 may lead to gene activation (59), while its interaction with HDAC1 may lead to repression (60,61). Our reporter assay confirms its function as a positive regulator of Gro-mediated repression, as knocking down Rm62 resulted in attenuated Gro activity. Interestingly, Rm62 was also shown to be an essential splicing component through its action on the U1 snRNP (62,63). The possible significance of the spliceosome in Gro mediated repression is discussed below.

An unanticipated role for the spliceosome in Gro mediated repression–One of the most surprising findings from our proteomic screen was the purification of a significant portion of the spliceosome complex, which suggests a potential role for the spliceosome in transcriptional regulation.

Pre-mRNA processing frequently occurs co-transcriptionally (64-66). Splicing factors are often recruited to nascent transcripts by the C-terminal domain (CTD) of the RNA Pol II large subunit and elongation factors (67,68). In addition, there is evidence that co-activators are able to interact with splicing factors (27). The interaction between the transcriptional and splicing machinery may be functionally relevant since different promoters can yield transcripts that are subject to differential alternative splicing (69,70). While many studies have focused on the effect of transcription factors in splicing, there is also increasing evidence that promoter proximal