U1 snRNA (a component of U1 snRNP). The results show that ~13% of the U1 snRNA in the nuclei of 0-12 hour embryos is associated with Gro (Figure 2B).

Functional analysis of Gro interacting proteins–We next carried out functional assays to determine if the interacting proteins are required for regulation of a Gro-responsive reporter gene. Previous studies established a reliable reporter assay for Gro function employing a luciferase reporter containing Gal4 binding sites (UAS elements), as well as an artificial enhancer containing binding sites for the Dorsal and Twist activators (14,16,18,47). Dorsal/Twist activated transcription of this reporter is strongly repressed upon introduction of a Gal4-Gro fusion protein. By altering the position of UAS elements relative to the artificial enhancer, we were able to examine both short-range and long-range Gro-mediated repression simultaneously (Figure 3A, B). The reporter system relied on two variants of click beetle luciferase that use D-luciferin as a substrate and emit either red or green light (48). In addition, a plasmid encoding Renilla luciferase, which uses coelenterazine as a substrate, was used as an internal control for transfection efficiency, cell viability, and general effects on transcription and translation. We validated the three-reporter system using dsRNA against Dorsal, Gro, and Rpd3 (which is partially required for Gro-mediated repression (18)) (Figure 3C). As predicted, Dorsal knockdown resulted in a complete loss of activation, Gro knockdown resulted in a complete loss of repression, and Rpd3 knockdown resulted in a partial loss of repression.

Each of the candidates from the screen for Gro-interacting proteins was knocked down by RNAi using up to three dsRNAs per gene to guard against off-target effects. We excluded the histones from this analysis under the assumption that knockdown of these essential chromatin components would have pleiotropic deleterious effects on cell metabolism, and because each histone is encoded by multiple genes making efficient knockdown problematic. We therefore tested 157 genes in this S2 cell luciferase assay, in most cases with multiple dsRNAs per gene (three if available), and each dsRNA was tested in triplicate. In total, we carried out approximately 1300 assays (including controls) in a 96 well plate format using a partially automated approach (see Experimental Procedures).

A candidate was scored as a regulator of Gro-mediated repression if knockdown reproducibly resulted in either an increase or a decrease in the level of repression (see Experimental Procedures for explanation of the statistical test of significance). Forty-four candidates met these criteria, of which 28 interfered with optimal repression (i.e., repression increased upon knockdown; these were termed "negative regulators of Gro") and 16 were required for optimal repression (i.e., repression decreased upon knockdown; these were termed "positive regulators of Gro"). We provide representative data for one negative regulator (vir), one positive regulator (snRNP-U1-C, and one protein that is neither a positive nor a negative regulator (Figure 3D); a list of all the positive and negative regulators (Table 5); and a separate list showing the quantitative effect of RNAi knockdown of each of the 44 regulators on repression by Gal4-Gro (Table S2). Of particular interest, four spliceosomal proteins, including two components of U1 snRNP, act as positive regulators of Gro, confirming the functional significance of the interaction between Gro and U1 snRNP. A few other noteworthy examples among the Gro regulators (Tables 5 and S2) include both components of the CKII complex (CKIIα, CKIIβ), which act as negative regulators, and the chromatin remodeling factor Acf1, which acts as a positive regulator (see discussion)

Expression profiling of Gro and snRNP-U1-C knockdown cells–snRNP-U1-C is one of the components of the U1 snRNP complex, which is responsible for 5' splice site recognition (46). In addition to its role in RNA processing, it has been