specificity of Gro-mediated repression and to reduced viability (19). To identify possible regulatory partners of these domains, we used them as affinity reagents to purify interacting proteins, which were then identified by mass spectrometry. The three central domains of Gro were expressed as glutathione-S-transferase (GST)-tagged proteins and purified from E. coli lysates (Figure 1A, B). We also constructed a similarly tagged form of the N-terminal Q domain since previous studies suggested that, in addition to mediating Gro oligomerization, the Q domain engages in interactions with regulatory targets (39,40).

The glutathione bead-immoblized GST-fused domains (or, as a negative control, immobilized unfused GST) were incubated with a Drosophila embryo nuclear extract. After extensive washing, interacting proteins were eluted with 2 M salt and analyzed by multi-dimensional protein identification technology (MudPIT) (33) (Table S1C). Duplicate extract preparations and affinity purifications were carried out and analyzed on separate dates and there was a high degree of overlap between the sets of proteins identified in these duplicate experiments (Figure 1C). With three exceptions (see Experimental Procedures), only proteins that appeared in both replicates were included in our list of Gro interacting proteins (Figure 1C, Table S1A, B.) Gene ontology analysis of this list of 162 proteins revealed a variety of functions including regulation of gene expression, RNA processing, and developmental processes (Table 3).

89 the 162 Gro-interacting proteins associated uniquely with one domain (in all but one case, the SP domain), while 32 interacted with two domains. In the case of 23 of the 32 proteins that interacted with two domains, one of these domains was the Q domain (Table S1A). This is consistent with the known role of the Q domain in homo-oligomerization (12-15). In accord with this role, chromatography using GST-Q as the affinity reagent resulted in the purification of some full-length endogenous Gro (Table S1C and data not shown). This could lead to the co-purification of Gro-interacting proteins that bind to regions outside the Q domain. Thus, 112 (89 plus 23) of the 162 detected interacting proteins can, in principal, be accounted for by the binding of Gro to a single central domain. However, at least 50 proteins (162 minus 112) are able to bind independently to two or three central domains. The ability to interact with multiple Gro domains could allow tighter binding or more versatile control of binding.

The list of interacting proteins (Table 4, Table S1A, B) contains multiple components of known multisubunit protein complexes. For example, we identified the α and β subunits of casein kinase II (CKII), a previously identified regulator of Gro activity (21). We also detected protein complexes involved in chromosome organization, including both components of the ATP-dependent chromatin remodeling and assembly factor (ACF), Acf1 and Iswi (41). Our proteomic screens also identified all the core protein components of the nucleosome (the core histones) as well as histone variant H2Av, consistent with previous studies demonstrating functional interactions between Gro and nucleosomes (42-44).

Perhaps most surprisingly, we discovered a number of components of the spliceosome among the group of Gro-interacting proteins, including all three proteins unique to U1 snRNP, components of U4/U6 snRNP, U2 snRNP, and the Sm complex (45,46). To validate the interaction between Gro and U1 snRNP, Drosophila embryo nuclear extracts were subjected to immunoprecipitation using an affinity purified antibody against the Gro GP domain or, as a negative control, rabbit IgG. An anti-Gro immunoblot of the immunopreciptated material demonstrates the efficiency of the immunoprecipitation (Figure 2A). RNA was extracted from the immunoprecipitates and analyzed by RT-qPCR with primers specific for