regulation in addition to its roles in RNA processing (26-30).

EXPERIMENTAL PROCEDURES

Plasmids–To generate plasmids for expression of GST fusion proteins, sequences encoding the Gro domains were amplified by PCR and inserted between the BamHI and XhoI sites of pGEX4T (GE Healthcare Life Sciences). The Q domain included Gro amino acids 1-133; the GP domain included amino acids 134-194, the CcN domain included amino acids 195-257, and the SP domain included amino acids 258-390. Sequences of PCR primers are provided in Table 1.

Plasmids used in the reporter assay were generated as follows. The red luciferase plasmid, G5DE5-pCBR, was generated by inserting the G5 DE5 enhancer region (14) into pCBR-basic vector (Promega Cat.# E1411) between the KpnI and XhoI sites. The green luciferase plasmid, DE5G5-pCBG68, was generated by inserting the luciferase gene using NcoI and SalI from pCBG68-basic vector (Promega Cat.# E1431) into the DE5 G5 vector, which has UAS elements downstream of the reporter (unpublished data). Actin promoter driven Dorsal (pPac Dl), Twist (pPac Twi) and Gal4-Gro (pAct Gal4-Gro) plasmids have been previously described (14). The RpIII128 promoter driven Renilla luciferase plasmid, RpIII128-Rluc, was obtained from Addgene (ID #37380) (31).

Affinity purification and identification of Gro interacting proteins–Plasmids encoding the recombinant domains fused to GST or GST alone were transformed into BL21 cells. 250 ml of mid-log cells were induced with 0.25 mM IPTG for an hour. Cells were pelleted at 4000 x g, resuspended in 25 mL of Salty TE (0.15 M NaCl, 10 mM Tris pH8, 1 mM EDTA) with protease inhibitor (Life Technologies, Cat.# 88266), and incubated on ice for 30 min. Samples were incubated at 4˚ for 15 min after DTT and Triton X-100 were added to final concentrations of 5 mM and 1%, respectively. Cells were then disrupted through a microfluidizer (Microfluidics M110L) using standard conditions. The lysate was collected and centrifuged at 14000 x g for 10 min at 4˚. Supernatant was collected, and 1 ml of glutathione agarose resin (50% slurry) was added. After overnight incubation, the resin was washed with cold PBS three times and stored at 4˚.

Drosophila embryo nuclear extracts were prepared as previously described (32). To isolate Gro-interacting proteins, 20 μg of glutathione bead-immobilized recombinant domains were mixed with nuclear extract containing 30 mg of protein (20 mg/ml) in 8 ml of HEMNK buffer (40 mM HEPES pH 7.5, 5 mM MgCl2, 0.2 mM EDTA, 1 mM DTT, 0.5% NP-40, 0.1 M KCl) at 4˚ overnight. Samples were washed six times for 15 minutes with 5 ml of HEMNK buffer. Proteins were first eluted with 5 ml of 2 M NaCl in HEMNK buffer and then with 2.5 ml of 2 M NaCl in HEMNK buffer for 20 min each. Eluted proteins were subjected to TCA precipitation prior to MudPIT analysis. MudPIT analysis was performed as previously described (33). Peptide identifications were filtered using a false discovery rate (FDR) cutoff of 0.05 as determined by the decoy database approach. Protein-level false positive rates were less than 0.03 for all individual runs.

Table S1C includes all the mass spectroscopy data for the two independent replicate screens carried out with each GST fusion protein and GST alone, while Tables S1A and B include selective data for 159 proteins that were detected in both replicates, as well as three proteins (Histone H3, Caf1, and bic) that were only detected in one replicate, but for which other data confirm the significance of the interaction (see notes 2 and 3 to Table S1B). Ribosomal proteins were excluded from the lists in Tables S1A and B.

Gro immunoprecipitation and reverse transcriptase qPCR (RT-qPCR) analysis of U1 snRNA–500 μg of nuclear extract was incubated with 1.875 μg of affinity purified rabbit antibody against the Gro GP domain or rabbit IgG in a final