Transcriptome Profiling Identifies Multiplexin as a Target of SAGA Deubiquitinase Activity in Glia Required for Precise Axon Guidance During Drosophila Visual Development

Jingqun Ma,* Kaelan J. Brennan,* Mitch R. D’Aloia,* Pete E. Pascuzzi,*‡,1 and Vikki M. Weake*§,1

*Department of Biochemistry, †Purdue University Libraries, and ‡Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907

ORCID IDs: 0000-0002-0079-6323 (J.M.); 0000-0002-9316-4404 (P.E.P.); 0000-0002-5933-9952 (V.M.W.)

ABSTRACT The Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex is a transcriptional coactivator with histone acetylase and deubiquitinase activities that plays an important role in visual development and function. In Drosophila melanogaster, four SAGA subunits are required for the deubiquitination of monoubiquitinated histone H2B (ubH2B): Nonstop, Sgf11, E(y)2, and Ataxin 7. Mutations that disrupt SAGA deubiquitinase activity cause defects in neuronal connectivity in the developing Drosophila visual system. In addition, mutations in SAGA result in the human progressive visual disorder spinocerebellar ataxia type 7 (SCA7). Gial cells play a crucial role in both the neuronal connectivity defect in nonstop and sgf11 flies, and in the retinal degeneration observed in SCA7 patients. Thus, we sought to identify the gene targets of SAGA deubiquitinase activity in glia in the Drosophila larval central nervous system. To do this, we enriched glia from wild-type, nonstop, and sgf11 larval optic lobes using affinity-purification of KASH-GFP tagged nuclei, and then examined each transcriptome using RNA-seq. Our analysis showed that SAGA deubiquitinase activity is required for proper expression of 16% of actively transcribed genes in glia, especially genes involved in proteasome function, protein folding and axon guidance. We further show that the SAGA deubiquitinase-activated gene Multiplexin (Mp) is required in glia for proper photoreceptor axon targeting. Mutations in the human ortholog of Mp, COL18A1, have been identified in a family with a SCA7-like progressive visual disorder, suggesting that defects in the expression of this gene in SCA7 patients could play a role in the retinal degeneration that is unique to this ataxia.

Chromatin regulators play an important role in neuronal development through their effects on gene expression. In particular, the removal of ubiquitin from monoubiquitinated histone H2B (ubH2B) is required for proper visual development (Poeck et al. 2001; Weake et al. 2008), and defects in histone deubiquitination are associated with retinal degeneration (David et al. 1997). Deubiquitination of ubH2B is catalyzed by the ubiquitin protease subunit of the Spt-Ada-Gcn5 Acetyltransferase (SAGA) transcriptional coactivator complex: Nonstop (FBgn0013717) in Drosophila melanogaster, Ubp8 in Saccharomyces cerevisiae, and USP22 in humans (Zhao et al. 2008; Henry et al. 2003; Weake et al. 2008; Zhang et al. 2008). SAGA’s ubiquitin protease requires three additional proteins for activity: Sgf11 (FBgn0036804), E(y)2, and ATXN7/Ataxin 7 (Kohler et al. 2010; Samara et al. 2010; Lang et al. 2011). Mutations in nonstop and sgf11 disrupt photoreceptor axon targeting in Drosophila (Weake et al. 2008; Poeck et al. 2001; Martin et al. 1995; Berger et al. 2008). In humans, polyglutamine (polyQ) expansion in ATXN7 results in SCA7, a dominant neurodegenerative disorder distinguished from other ataxias by retinopathy (David et al. 1997; Enevoldson et al. 1994). Notably, polyQ-expanded ATXN7 reduces SAGA deubiquitinase activity in vivo; thus, defects in ubH2B deubiquitination could induce the retinal degeneration that is unique to this ataxia (McCullough et al. 2012; Yang et al. 2015; Lan et al. 2015).

KEYWORDS histone ubiquitination glia axon guidance SAGA SCA7
Although SAGA associates with and deubiquitinates ubH2B at the majority of actively transcribed genes, only a subset of these genes require ubH2B deubiquitination for expression (Weake et al. 2011; Bonnet et al. 2014). Studies in yeast and flies indicate that SAGA deubiquitinase activity is required for activation of inducible, tissue-specific genes that regulate developmental processes (Henry et al. 2003; Weake et al. 2011). However, the SAGA-regulated genes that are required for visual development, and that directly result in the visual degeneration in SCA7 patients, are unknown.

Several studies indicate that proper SAGA deubiquitinase activity is required in glia, rather than neurons, for both visual development and healthy eye function. Clonal analysis indicates that nonstop is required in glia for their migration to the correct region of the brain where they subsequently provide appropriate termination cues to photoreceptor axons (Poeck et al. 2001). In addition, expression of polyQ-expanded ATXN7 in glia is sufficient to induce neurodegeneration in a SCA7 mouse model (Custer et al. 2006). Thus, we sought to identify genes regulated by SAGA deubiquitinase activity in glia, with the aim of identifying genes that are required in glia for proper migration, and that could lead to visual degeneration in SCA7.

Previously, we attempted to identify SAGA deubiquitinase-regulated genes that are required for neuronal connectivity by microarray analysis of gene expression in larvae (Weake et al. 2008). However, this approach did not identify suitable candidate genes due to the small number of lamina glial cells in the whole larvae. Here, we characterize the SAGA deubiquitinase-dependent transcriptome of glia enriched from the central nervous system and eye-antennal imaginal disc of Drosophila third instar larvae, and identify Multiplexin (Mp) as a target of SAGA deubiquitinase activity that is required in glia for lamina glial organization and proper photoreceptor axon targeting.

**MATERIALS AND METHODS**

**Genetics**

The nonstop and sgf11 alleles used in this study were previously described (Weake et al. 2008). Flies expressing the UAS-KASH-GFP transgene, P[w+mC = UAS-GFP-Msp300KASH]attP2, were previously described (Ma and Weake 2014). The following three genotypes were used to label glial nuclei for affinity-enrichment in wild-type, nonstop, or sgf11 mutant larvae respectively: w;P[w+mC = GAL4]repo, P[w+mC = UAS-GFP-Msp300KASH]attP2/TM3, Sb2 for wild type; w;P[w+mC = GAL4]repo, P[y+ts7.2 = PZ]noto12085; P[y+ts7.2 = PZ]noto12085; P[w+mC = UAS-GFP-Msp300KASH]attP2 for nonstop, and w;P[w+mC = GAL4]repo, Pbac[w+mC = RB]CG1337901308/ Pbac[w+mC = RB]CG1337901308, P[w+mC = UAS-GFP-Msp300KASH]attP2 for sgf11. For RNAi crosses, w;UAS-dicer2; P[w+mC = GAL4]repo, ro-rlacZ/TM6C, Tb, Sb flies were crossed with the following RNAi stocks provided by the Bloomington Drosophila Stock Center at Indiana University (Luciferase: BL35788; Mph: BL52981, BL32921; Jing: BL55633, BL72024; Rab6: BL27490, BL35744; ras: BL31653, BL31654; and uzip: BL29558) and the Vienna Drosophila RNAi center (sgf11: 17166). RNAi crosses were performed at 28° and wandering third instar larval F1 progeny were analyzed. The following two genotypes were used for analysis of loco"C"6 localization in wild-type or sgf11 larvae: w;P[y+ = lacZ-uni1]locos"C6", Pbac[w+mC = RB]CG1337901308 and w;P[y+ = lacZ-uni1]locos"C6" (BL10009) (Granderath et al. 1999; Winberg et al. 1992).

**Immunohistochemistry and X-gal staining**

Central nervous system/eye-antennal disc lobe complexes from wandering third instar larvae were dissected and fixed with 4% formaldehyde before immunostaining with the following antibodies: anti-chaoptin (mAb24B10, mouse, 1:10; Developmental Studies Hybridoma Bank) (Fujita et al. 1982); anti-repo (8D12, mouse, 1:10, Developmental Studies Hybridoma Bank) (Alfonso and Jones 2002); anti-β-galactosidase (#A11132, rabbit, 1:500, Molecular Probes); goat anti-mouse Alexa Fluor 568 (#A10004, 1:300, Life Technologies); and goat anti-rabbit Alexa Fluor 488 (#A11001, 1:300, Life Technologies). Nuclei were stained using 0.1 µg/ml 4,6-diamidino-2-phenylindole (DAPI, #40011, Biotium). Laser scanning confocal imaging was performed using a Nikon A1R inverted confocal microscope under a 40 × 1.30 NA oil immersion Nikon Plan Fluor objective. Confocal images are presented either as single planes or as 3-D maximum projection images consisting of 0.5–1.0 µm z-stacks using NIS-Elements software. X-gal staining of central nervous system/eye-antennal disc complexes from wandering third instar larvae was performed as described (Sweeney et al. 2012) with the following modifications: dissected central nervous system/eye-antennal disc complexes were fixed in 1% formaldehyde prior to staining, and 0.3% Triton X-100 was included in the X-gal staining solution. Stained complexes were examined using a Zeiss Discovery V12 light microscope.

**Gli nuclear RNA isolation and RNA sequencing (RNA-seq)**

Central nervous system/eye-antennal disc complexes were dissected from wild-type, nonstop, and sgf11 wandering third instar larva. GFP-labeled glial nuclei were enriched from 400 dissected eye-brain complexes for each biological replicate and genotyped as previously described (Ma and Weake 2014). Total nuclear RNA was extracted from isolated glial nuclei using Trizol reagent (Life Technologies), treated with DNase (Roche), and mRNA was enriched and purified using an RNeasy MinElute Cleanup Kit (#74204, QIAGEN). RNA (7 ng) was used to generate double-stranded cDNA for each sample using the Ovation RNA-Seq System V2 (#7102, NuGEN technologies). Downstream indexed TruSeq PCR-free DNA libraries (Illumina) were constructed from amplified, double-stranded cDNA. All 12 samples were added to a single pool that was clustered in two lanes of a HiSeq Instrument 2500 paired-end v3 high output flowcell to generate two 101 base reads per cluster.

**RNA-seq data analysis**

Four biological replicates were analyzed for each of the following genotypes: wild type, nonstop, and sgf11. Quality trimming was performed on paired-end reads for all 12 samples using Trimmomatic (v0.32) (Bolger et al. 2014) to remove bases with Phred33 < 30, resulting in properly paired reads of at least 50 bases. Quality trimmed reads were mapped against the bowtie-2 (v2.2.4) (Langmead and Salzberg 2012) indexed D. melanogaster genome (Drosophila_melanogaster. BDGP5.78) using TopHat (v2.0.13) (Trapnell et al. 2009). The raw counts matrix was generated by Htsseq-count (v0.6.1) applying no strand-specific assay, union mode, and default parameters (Anders and Huber 2010). Differential expression analysis was performed on genes with greater than one count per million (CPM) in at least four of the 12 samples. Differentially expressed genes were detected in each mutant genotype relative to the wild-type genotype using edgeR (Robinson et al. 2010) using a False Discovery Rate (FDR) of less than 0.01. The distance matrix and scatter plots were generated using Bioconductor packages of DESeq2 (Love et al. 2014) and edgeR (Robinson et al. 2010), respectively, in R (v3.1.2). Gene Ontology (GO) term enrichment analysis was performed using a Fisher’s exact test and significantly enriched GO terms were defined as those with a FDR < 0.001. Actively transcribed genes were defined as fragments per
kilobase of transcript per million mapped reads (FPKM) of greater than one in wild-type glia. The GO term analysis used the Bioconductor *Drosophila* genome annotation package 3.1.2 with GO data from March 17, 2015. GO terms with less than eight or more than 250 genes were removed, as were gene annotations with no supporting data.

The RNA-seq data for the central nervous system of OregonR larvae were obtained from the modENCODE project: Dm Tissue Expression RNA-seq third instar larvae central nervous system sequences (ModENCODE_4257). Two biological replicates for larval central nervous system RNA-seq data were mapped against the bowtie-2 (v2.2.4) (Langmead and Salzberg 2012) indexed D. melanogaster genome (Drosophila_melanogaster.BDGP5.78) using Tophat (v2.0.13) (Trapnell et al. 2009). A raw counts matrix was generated by Htsseq-count (v0.6.1) applying no strand-specific assay, union mode, and default parameters (Anders and Huber 2010). A count matrix for the central nervous system and the wild-type glia nuclei was assembled, and edgeR was used to normalize libraries and determine the FPKM values for genes that had greater than one CPM in two or more of the six samples.

### qRT-PCR analysis

Exonic primers flanking intron regions of target genes were designed for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis using Primer3. qRT-PCR analysis was performed on cDNA as previously described (Ma and Weake 2014).

### Data availability

All *Drosophila* strains are available upon request. The RNA-seq expression data discussed in this publication have been deposited in NCBI’s Gene Expression Omnibus (Edgar et al. 2002) and are accessible through GEO series accession number GSE75681 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75681). RNA-seq data for the central nervous system of OregonR larvae were obtained from the modENCODE project: Dm Tissue Expression RNA-seq third instar larvae central nervous system sequences (ModENCODE_4257: SRR070409 and SRR070410). FPKM values for central nervous system vs. glial-enriched samples are reported in Supplemental Material, Table S1. Lists of up- and downregulated genes for each mutant relative to the wild type are provided in Table S2, Table S3, Table S4, Table S5, Table S6, and Table S7. Complete edgeR analysis results are reported in Table S8 and Table S9. Primer sequences are listed in Table S10. Raw counts for RNA-seq data are provided in Table S11. All R Code used in this study is available upon request.

### RESULTS

#### Enrichment of glia from wild-type, nonstop, and sgf11 optic lobes for transcriptome profiling

The compound eye of *Drosophila* is composed of ~800 ommatidia, each of which contain eight different photoreceptor neurons (R1–R8 cells) arranged in a stereotypical pattern. During the third larval instar, R1–R8 photoreceptors extend axons from the eye imaginal disc through the optic stalk where they project into different synaptic layers in the optic lobe: R1–R6 project into the lamina between two layers of glial cells, the epithelial and marginal glia, while R7 and R8 extend further into the medulla (Clandinin and Zipursky 2002). Mutations in *nonstop* and *sgf11* result in a failure of glial cells to migrate into the lamina and misprojection of R1–R6 axons into the medulla (Poeck et al. 2001; Weake et al. 2008). Since clonal analysis indicates that the SAGA deubiquitinase *Nonstop* is required in glia to regulate cell migration (Poeck et al. 2001), we hypothesized that SAGA deubiquitinase activity is required in glia for the expression of specific genes that regulate glial migration, which in turn controls proper photoreceptor axon targeting. However, we note the formal possibility that SAGA could also be required in glia to provide the termination signal necessary for proper axon targeting; this alternative hypothesis will be addressed later in this study.

As a first step in identifying novel SAGA-regulated genes that are required in glia for neuronal targeting, we sought to identify genes regulated by SAGA deubiquitinase activity, specifically in optic lobe glia. To do this, we utilized our previously developed nuclei affinity purification protocol to enrich glial nuclei from the eye imaginal disc and optic lobes of wild-type, *nonstop*, and *sgf11* third instar larvae for transcriptome profiling (Ma and Weake 2014). This protocol utilizes antibodies coupled to magnetic beads to affinity enrich nuclei labeled with GFP fused to the Klarsicht, Anc-1, and Syn3-1 homology (KASH) domain of Msp300, which localizes EGFP to the cytoplasmic face of the nuclear membrane and is expressed under Gal4/USAS control (UAS-KASH-GFP) (Yu et al. 2006; Patterson et al. 2004; Fischer et al. 2004). To label glial nuclei with KASH-GFP, we crossed flies carrying the UAS-KASH-GFP transgene with the glial-specific repo-Gal4 driver (Xiong et al. 1994; Halter et al. 1995; Sepp et al. 2001). To label glial nuclei in *nonstop* and *sgf11* mutant larvae with KASH-GFP, we crossed flies carrying the *nonstop* or *sgf11* mutant allele on the same chromosome as repo-GAL4 to flies with the mutant allele on the same chromosome as UAS-KASH-GFP, as described by the genetic scheme outlined in Figure 1A. Using this approach, only glial nuclei in homozygous *nonstop* or *sgf11* mutant larvae are labeled with KASH-GFP. We do not obtain homozygous *nonstop* or *sgf11* adult flies since these mutations result in late larva/early pupal lethality.

To test if glial nuclei were successfully labeled with KASH-GFP in each genotype, we examined the optic lobes dissected from third instar larvae using confocal microscopy. We immunostained the dissected eye-brain complexes with anti-chaoptin, which labels R1–R8 photoreceptor axons, to examine photoreceptor projections for comparison in each genotype. In optic lobes dissected from third instar larvae expressing UAS-KASH-GFP under repo-Gal4 control, we observe GFP surrounding a DAPI-positive region in glial cells, consistent with nuclear envelope localization of the KASH-GFP tag (Figure 1B, arrow). Notably, we only observe GFP-labeled glia in a subset of the larval progeny from the *nonstop* or *sgf11* crosses, as expected from the genetic scheme described in Figure 1A. Further, progeny from the *nonstop* or *sgf11* crosses that exhibit GFP-labeled glia also show defects in photoreceptor axon targeting when compared with the wild type (Figure 1, C–E). Whereas in wild-type optic lobes, photoreceptor axons form thick growth cones in the lamina plexus (Figure 1C), in *nonstop* and *sgf11* optic lobes, many photoreceptor axons project through the lamina and terminate improperly in the medulla (Figure 1, D–E). Thus, glial nuclei were positively labeled with KASH-GFP in optic lobes from each of the wild-type, *nonstop*, and *sgf11* genotypes, enabling us to subsequently enrich these labeled nuclei using affinity purification (Ma and Weake 2014).

#### Enriched glial nuclei express higher levels of glial-specific gene markers relative to the whole central nervous system

Since glial cells constitute no more than 10% of the total cells present in the central nervous system (Edwards and Meinertzhagen 2010), we sought to determine the level of enrichment of glial-expressed genes relative to the entire larval central nervous system in our affinity enriched nuclei. Initially, we examined levels of GFP transcripts in samples pre- and postaffinity enrichment, and found that GFP levels...
were 10–30 fold higher in glial nuclei from wild-type larvae following affinity enrichment (Figure S1), indicating that our samples are enriched for the GFP-labeled nuclei population of interest.

To estimate the enrichment of RNAs for glial-specific genes in our isolated nuclei, we compared gene expression in our wild-type glial samples to gene expression for the larval central nervous system. The libraries were normalized and gene expression (FPKM) was estimated with edgeR (Table S1). The expression level of genes that have previously been shown to be preferentially expressed in either glia or neurons was examined. The glial marker repo is expressed at ~fourfold higher levels in the wild-type glia samples compared to the central nervous system (Figure 1F). However, four other glial-specific genes, spin, moody, dawdle (daw), and Multi drug resistance 65 (Mdr65), show much higher enrichment ranging from 9–68-fold (Bainton et al. 2005; Mayer et al. 2009; Zhu et al. 2008; Yuva-Aydemir et al. 2011). In contrast, several other glial marker genes including nervana 2 (nrv2), locomotion defects (loco), Glutamine synthetase 2 (Gg2), and G protein α i subunit (Goi) are enriched between two- and threefold (Thomas and van Meyel 2007; Granderath et al. 1999; Freeman et al. 2003; Pereanu et al. 2005; DeSalvo et al. 2014). We note that the larval central nervous system RNA-seq data used for this comparison represents polyadenylated mRNA isolated from bulk tissue. Since our approach examines nuclear RNA rather than total cellular RNA, it is possible that the different levels of enrichment observed for these glial markers could
represent temporal differences in transcription relative to total cellular mRNA levels. Other studies that have compared active transcription with steady-state mRNA by identifying intron regions (irRNA-seq) or nascent transcripts (GRO-seq) have shown that there is a lag of minutes to a few hours between acute changes in transcription and detectable changes in steady-state mRNA levels (Madsen et al. 2015; Step et al. 2014).

Next, we examined the expression level of several genes that have been shown to be expressed in neurons or their precursors. The well-characterized neuronal marker elav shows a twofold reduction in expression levels in the enriched-glial samples relative to the whole central nervous system (Figure 1G) (Robinow and White 1991). In addition, the transcription factor gom2, which is expressed in glial and neuronal progenitor cells, shows an eightfold reduction in expression (Chotard et al. 2005). Other neuronal markers such as β amyloid protein precursor-like (Aphl), jelly belly (jeb), Netrin-A (NetA), roundabout 3 (robo3), camaruff (erm), single-minded (sim), and Anaplastic lymphoma kinase (Alk) show a 3–10-fold reduction in expression levels relative to the central nervous system (Martin-Morris and White 1990; Pecot et al. 2014; Bazigou et al. 2007; Timofeev et al. 2012; Pappu et al. 2011; Umetsu et al. 2006; Weng et al. 2010). In addition, the lamina neuron markers dachshund (dac) and pou domain motif 3 (pdm3) are reduced by twofold in the enriched-glial samples relative to the whole central nervous system (Mardon et al. 1994; Huang and Kunes 1996; Chen et al. 2012). We note that a recent study has shown that pdm3 is also expressed in perineurial glial cells in the eye imaginal disc, possibly accounting for the relatively high expression of this gene in the glial-enriched data (Bauke et al. 2015). Surprisingly, we observed an enrichment rather than reduction in expression of the neuroblast marker gene, seven up (svp), in the glial-enriched samples relative to the central nervous system (Chang et al. 2003). However, there is data supporting expression of svp in a subset of glial cells in embryos, suggesting that this gene might also be expressed in glial cells in the central nervous system (Beckervordersandforth et al. 2008).

Based on the direct comparison of GFP expression in our glial-enriched sample to the bulk nuclei prior to affinity enrichment (pre-isolation sample, Figure S1), we conclude that we have enriched the proportion of glial RNAs ~10-fold relative to the entire central nervous system. Comparison of the glial-enriched data with publicly available RNA-seq data for the larval central nervous system supports an enrichment of glial-specific RNAs and a reduction in the level of contaminating neuronal RNAs. However, since there are major differences in these data sets, in particular with regard to total cellular RNA vs. nuclear RNA, this comparison only provides a rough estimate of the level of enrichment of glial RNAs in our samples.

Transcriptome profiling reveals genes that are coregulated by Nonstop and Sgf11 in glia

To identify genes that are transcriptionally regulated by SAGA deubiquitinase activity in glia, we isolated RNA from the enriched glial nuclei in nonstop and sgf11 optic lobes and compared these transcriptomes with that of the wild type using RNA-seq. Four biological replicates were conducted for each genotype. We calculated the Euclidean distance matrix based on log transformed count data. This analysis showed that the transcriptome profiling of nonstop and sgf11 optic lobe glia were similar to each other, and distinct from that of the wild-type glia (Figure 2A). The similarity of the nonstop and sgf11 expression profiles suggests that loss of Nonstop or Sgf11 has a similar effect on gene expression, consistent with the findings of previous microarray studies and with their joint function in SAGA-mediated ubH2B deubiquitination (Weake et al. 2008).

To identify genes that are transcriptionally regulated by SAGA deubiquitinase activity, we therefore sought to identify genes that are coregulated by Nonstop and Sgf11. To do this, we conducted edgeR analysis to identify genes that are misregulated in nonstop or sgf11 glia using a FDR of < 0.01 (Robinson et al. 2010). Using this approach, we identified 966 (Table S2) and 836 (Table S3) genes as significantly up- or downregulated, respectively, in the nonstop glia relative to wild-type glia, and 900 (Table S4) and 744 (Table S3) genes as significantly up- or downregulated, respectively, in the sgf11 glia relative to wild-type glia (Figure 2B). Consistent with the similarity of the nonstop and sgf11 expression profiles (Figure 2A), the majority of differentially expressed genes identified in nonstop and sgf11 glia were overlapping. We identified 779 (Table S6) genes as being significantly upregulated in both nonstop and sgf11 glia as compared to wild-type glia, and 629 (Table S7) genes as being significantly downregulated in both nonstop and sgf11 glia (Figure 2B). Further, when we examined the change in transcript level of the significantly up- or downregulated genes in each mutant genotype relative to the wild type, and plotted this against the expression level of each gene, we observed that most of the differentially expressed genes that were identified in only one mutant exhibited smaller fold changes relative to those genes that were coregulated by Nonstop and Sgf11 (Figure 2C). Hence, we chose to focus our further analysis on the commonly up- and downregulated genes identified in nonstop and sgf11 glia relative to wild-type glia. These Nonstop and Sgf11 coregulated genes will be hereafter referred to as SAGA deubiquitinase-regulated genes.

To validate the transcript level changes observed in our RNA-seq analysis, we analyzed transcript levels of 13 significantly downregulated genes and six significantly upregulated genes of potential biological relevance in wild-type, nonstop, and sgf11 glia by qRT-PCR. The transcript level of each gene was normalized to the ribosomal gene Rpl32, which is not transcriptionally regulated by SAGA (Weake et al. 2011). As expected from the RNA-seq analysis, all 13 of the significantly downregulated genes examined showed lower transcript levels in nonstop and sgf11 glia relative to wild-type glia. These Nonstop and Sgf11 coregulated genes will be hereafter referred to as SAGA deubiquitinase-regulated genes.

SAGA deubiquitinase activates expression of genes involved in proteasomal degradation, protein folding, and axon guidance

To elucidate the characteristics of the SAGA deubiquitinase-regulated genes in glia, we examined the 1408 SAGA deubiquitinase-regulated genes for enrichment of specific GO terms. To do this, we conducted overrepresentation analysis relative to the entire genome separately for the upregulated and downregulated gene lists using Fisher’s exact test with a FDR of 0.001. Using this approach, we identified two and 12 biological processes as being significantly enriched in SAGA deubiquitinase up- and downregulated genes, respectively (Figure 3). Intriguingly, SAGA deubiquitinase activity appears to be important for full expression of genes involved in the proteasome-mediated protein catabolic process. Strikingly, 29 out of the 50 genes annotated as proteasome subunits, including Regulatory particle non-ATPase 2 (Rpn2) (Figure 2D),
are downregulated in SAGA deubiquitinase mutant glia. In addition to protein degradation, SAGA deubiquitinase activity also positively regulates the expression of genes involved in protein folding. One of the most highly downregulated genes identified in SAGA deubiquitinase mutants relative to the wild type, Heat shock protein 27 (Hsp27), encodes a molecular chaperone required for proper protein folding (Figure 2D). In addition to these processes that play a critical role in glial function, we identified genes involved in axon guidance as being significantly enriched among the SAGA deubiquitinase downregulated genes.

**The SAGA deubiquitinase target Mp is required in glia for proper axon targeting**

To test the hypothesis that SAGA deubiquitinase activity regulates the expression of genes that are required in glia for proper photoreceptor axon targeting, we sought to identify SAGA-regulated genes that were expressed in glia with potential functions in controlling axon guidance. To do this, we compared the list of 629 genes that were significantly downregulated in SAGA deubiquitinase mutant glia (Table S7) with the 3701 genes that were expressed in wild-type optic lobe glia compared to wild-type optic lobe glia. (C) Scatter plots illustrating the average differential expression of each gene (dot) in mutant/wild-type glia relative to its average expression across all samples. Each gene is plotted based on the log2 expression ratio in either the nonstop (upper panel) or sgf11 (lower panel) mutant/wild type (y-axis) relative to the log2 of its average expression level in CPM across all genotypes (x-axis). Genes that were identified as being significantly differentially regulated in both mutant genotypes are shown in red, and genes identified as significantly differentially regulated only in a single mutant genotype are shown in blue. (D) qRT-PCR analysis of transcript levels in cDNA from wild-type, nonstop, and sgf11 optic lobe glia for a subset of the differentially regulated genes was compared with fold changes observed in the RNA-seq analysis. Mean transcript levels for each gene were normalized to RpL32 and plotted relative to the wild type, which was set to one (right panel). RNA-seq result with fold change of genes of interest were plotted using raw abundance values compared to the wild type, which was set to one (left panel). Error bars denote standard error of the mean for three biological replicates for qRT-PCR analysis and four biological replicates for RNA-seq. CPM, count per million; FDR, false discovery rate; qPCR, quantitative polymerase chain reaction; RNA-seq, RNA sequencing; SAGA, Spt-Ada-Gcn5 acetyltransferase; WT, wild-type.
not as highly expressed in glia relative to the central nervous system as compared with the other five genes. We expressed RNAi constructs in glia using the repo-Gal4 driver and examined photoreceptor axon targeting using the R2–R5-specific marker ro-\textit{lacZ} (Garry et al. 1996; Sepp et al. 2001). Axon targeting was examined for each RNAi line in the presence and absence of the Gal4 driver to control for nonspecific expression of RNAi. Axon targeting defects were classified based on the number of misprojected axons as either no defect (mistargeted axons ≤ 1; Figure 4B), mild defect (mistargeted axons ≤ 4; Figure 4C), or severe defect (mistargeted axons ≥ 5; Figure 4D). As controls, we examined photoreceptor axon targeting upon expression of RNAi against \( \textit{sgf11} \) and the nonspecific gene, \textit{Luciferase}. Glial-specific expression of RNAi against \( \textit{sgf11} \) results in mild to severe axon targeting defects in 74% of optic lobes analyzed (Figure 4E). In contrast, knockdown of \textit{Luciferase} resulted in only 3 out of 40 analyzed optic lobes showing a mild axon targeting defect (Figure 4E). Out of the five genes we examined, only one gene showed a significant defect in photoreceptor axon targeting upon glial-specific expression of RNAi relative to the minus Gal4 control for both independent RNAi lines: \textit{Multiplein} (\( \textit{Mpl} \)). Glial-specific expression of two independent RNAi constructs against \( \textit{Mpl} \) results in a mild to severe axon targeting defect in 52% and 84%, respectively, of the optic lobes analyzed, similar to the level of axon targeting defect observed upon expression of RNAi against \( \textit{sgf11} \) (Figure 4E). \( \textit{Mpl} \) had previously been shown to be required in neurons for proper motor axon targeting (Meyer and Moussian 2009). Our results indicate that \( \textit{Mpl} \) is also required noncell-autonomously in glia for proper neuronal targeting. Although we were only able to identify a requirement for \( \textit{Mpl} \) in glia for photoreceptor axon targeting in the genes analyzed in this study using our strict criteria, we note that one of the other genes we examined, \textit{jing}, showed an extremely severe axon targeting defect for one (but not both) RNAi lines tested.

**Glia-specific knockdown of \( \textit{Mpl} \) modestly disrupts lamina glial organization, which correlates with mistargeting of photoreceptor axons**

The prevailing model for how photoreceptor axons R1–R6 find their targets in the brain is that lamina glial cells provide the signals that are necessary to control photoreceptor axon termination (Poeck et al. 2001). Since both \( \textit{sgf11} \) and \textit{nonstop} mutant larvae have strong defects in glial migration (Weake et al. 2008; Poeck et al. 2001), the simplest model for how SAGA functions in this process is that SAGA deubiquitinase activity is required for the expression of genes in glia that are necessary for their proper migration. However, one alternative possibility is that SAGA deubiquitinase-activated genes are also required in glia following migration to provide the appropriate termination signal to photoreceptor axons. While this second possibility is unlikely, neither we nor Poeck et al. (2001) were previously able to distinguish whether SAGA was also required in glia, following migration, to provide the termination cue necessary for axon targeting. Thus, to address this second possibility, we reexamined glial organization in \( \textit{sgf11} \) homozygous mutant larvae using a \textit{lacZ} enhancer trap in the \textit{locomotion defects} locus (\textit{locorsg}5) that marks lamina glia, including both marginal and epithelial glial layers, medullary glial cells, and subretinal cells (Granderath et al. 1999; Winberg et al. 1992). In the wild type, a thick layer of lamina glial cells is observed in the lamina region \textit{(lia)} where R1–R6 photoreceptor axons terminate, forming the lamina plexus (Figure 5A). In contrast, significant numbers of these glia are absent from the lamina region in the \( \textit{sgf11} \) mutant, concomitant with misprojection of photoreceptor axons into the medulla (Figure 5A, boxed region R2). However, as shown in the \( \textit{sgf11} \) optic lobe in Figure 5A, in some mutant animals a subset of glia migrate appropriately to the lamina (Figure 5A, boxed region R1). Notably, in these regions of the lamina where glia are present, we observe normal photoreceptor axon termination. From this, we conclude that although SAGA deubiquitinase activity is required for glia to migrate, it is not subsequently required in glia to provide the signal necessary for proper axon termination. Based on these observations, we hypothesized that the photoreceptor axon targeting defects observed upon glial-specific expression of RNAi against the SAGA deubiquitinase-activated gene, \( \textit{Mpl} \), would correlate with defects in lamina glial migration. Thus, we examined both glial organization and axon targeting in optic lobes in which RNAi against \( \textit{Mpl} \) had been expressed specifically in glia. To visualize photoreceptor axons, we used \textit{ro-\textit{lacZ}}, which labels R2–R5 photoreceptor axons (Garry et al. 1996). To label glia, we immunostained with antibodies against the glial-specific transcription factor Repo (Xiong et al. 1994). As expected from our previous X-gal staining analysis (Figure 4), we observed reproducible axon targeting defects upon expression of RNAi against both \( \textit{sgf11} \) and \( \textit{Mpl} \) in glia (Figure 5, C–D, arrowheads). In contrast, glial-specific expression of RNAi against \textit{Luciferase} does not result in any observable axon targeting defect (Figure 5B), and well...
optic lobes in which entire regions of lamina glial cells are missing, expression of RNAi against \textit{sgf11} results in a slight disruption to glial organization (Figure 5C). Surprisingly, this modest defect in glial organization is still associated with mistargeting of a subset of photoreceptor axons (Figure 5C, arrowheads). It is likely that expression of RNAi against \textit{sgf11} does not result in complete loss of Sgf11 protein, however, we cannot confirm this since there is no antibody available to detect Sgf11. Thus, we conclude that knockdown of Sgf11 in glial cells modestly disrupts lamina glial cell migration and/or organization, and correlates with mistargeting of photoreceptor R1–R6 axons.

Similar to expression of RNAi against \textit{sgf11}, expression of RNAi against \textit{Mp} does not cause a significant defect in glial migration. However, we do observe modest disorganization of lamina glia (Figure 5D, arrowheads), and this slight disruption to lamina glial organization is sufficient to result in mistargeting of photoreceptor axons (Figure 5D, arrowheads). These observations show that there is a spatial correlation between defects in glial cell organization and axon mistargeting upon knockdown of \textit{Mp}, indicating that \textit{Mp} is required in glia for proper lamina glial migration and/or organization, which in turn controls photoreceptor axon termination. However, based on these analyses alone, we cannot formally exclude the possibility that \textit{Mp} is also required in glia to provide a termination signal to photoreceptor axons, even though SAGA itself is not.

**DISCUSSION**

Here, we identify novel gene targets of the transcription coactivator SAGA in \textit{Drosophila} central nervous system glia. We show that the SAGA-activated gene, \textit{Mp}, is required in glia for proper lamina glial organization and subsequent photoreceptor axon targeting in the optic lobe. Prior to this study, nonstop, \textit{sgf11}, and the miRNA \textit{bantam} were the only genes reported to be required in lamina glia for their migration (Li and Padgett 2012).

\textit{Mp} encodes an ortholog of vertebrate collagen XV/XVIII and consists of three major domains: an N-terminal thrombospondin-related domain, triple helix, and a C-terminal Endostatin domain. The identification of \textit{Mp} in this study suggests a novel role for collagen XV/XVIII in glial migration. \textit{Mp} had previously been shown to be required in neurons for correct motor neuron axon pathfinding during embryogenesis (Meyer and Moussian 2009). The C-terminal Endostatin domain of \textit{Mp}, which can be proteolytically released (Heljasvaara et al. 2005), is sufficient for proper motor axon pathfinding in \textit{Drosophila} embryos (Meyer and Moussian 2009). Endostatin, which acts as a signaling molecule (Wickstrom et al. 2005), is also necessary for homoeostatic synaptic plasticity, and modulates both presynaptic calcium influx and neurotransmitter release (Wang et al. 2014). Intriguingly, mutations in human collagen XVIII (\textit{COL18A1}) are associated with Knobloch syndrome, a rare autosomal recessive disorder characterized by severe vision problems including vitreoretinal degeneration (Sertie et al. 2000; Bishop et al. 2010). In addition, a mutation in \textit{COL18A1} has been identified in an Indian family with \textit{SCA7}-like symptoms including ataxia and progressive blindness (Paisan-Ruiz et al. 2009). Since polyQ-expanded h\textit{ATXN7} reduces SAGA deubiquitinase activity \textit{in vivo}, in part through sequestration of the ubiquitin protease USP22 (McCullough et al. 2012; Yang et al. 2015; Lan et al. 2015), SAGA deubiquitinase-regulated gene expression is likely to be defective in \textit{SCA7} patients. Notably, expression of polyQ-expanded \textit{ATXN7} in glia is sufficient to induce neurodegeneration in a \textit{SCA7} mouse model (Caster et al. 2006), indicating that SAGA deubiquitinase function in glial cells plays a crucial role in \textit{SCA7} pathogenesis. This finding is consistent with the general role that glia play in the progression, and in some cases initiation, of neurodegeneration in polyQ-diseases.
and targeting of photoreceptor axons was examined using the lamina ganglia where they express the locorC56 (mAB24B10; red). In WT larvae, glia migrate appropriately to the in WT and photoreceptor axon mistargeting. (A) Lamina glia were visualized modestly disrupts lamina glial organization, which correlates with mistargeting of photoreceptor axons. Merged images for glia and axons are shown in the upper panel, and single channel images for glia alone (left and middle panels, green). Glial cells were labeled for comparison using anti-repo marker. In locorC56 larvae, although some glia migrate appropriately and express locorC56 (boxed region R1), many glia are absent from the lamina (boxed region R2), correlating with mistargeting of photoreceptor axons. Merged images for glia and axons were expressed specifically in glia using the repo-GAL4 driver, and targeting of photoreceptor axons was examined using the R2–R5 photoreceptor axon marker or-tacZ (left and middle panels, green). Glial cells were labeled for comparison using anti-repo (middle and right panels, magenta). Maximum projection images of 0.5 μm z-stacks are shown for each knock-down. The positions of individual mistargeted photoreceptor axons are indicated by arrowheads in each panel. The expected position of lamina glial cells is indicated by dotted lines in the right panels for each genotype. Scale bars, 20 μm. RNAi, RNA interference; WT, wild-type.

Figure 5 Glial-specific expression of RNAi against Multiplexin modestly disrupts lamina glial organization, which correlates with photoreceptor axon mistargeting. (A) Lamina glia were visualized in WT and sgf11 optic lobes using the locorC56 marker (blue), and R1–R8 photoreceptor axons were labeled using anti-chaoptin (mAB24B10; red). In WT larvae, glia migrate appropriately to the lamina ganglia where they express the locorC56 marker. In sgf11 larvae, although some glia migrate appropriately and express locorC56 (boxed region R1), many glia are absent from the lamina (boxed region R2), correlating with mistargeting of photoreceptor axons. Merged images for glia and axons are shown in the upper panel, and single channel images for glia alone (left and middle panels, green). Glial cells were labeled for comparison using anti-repo marker. In WT and sgf11, glia correlate with mistargeting of photoreceptor axons.

(A) Lamina glia were visualized in WT and sgf11 optic lobes using the locorC56 marker (blue), and R1–R8 photoreceptor axons were labeled using anti-chaoptin (mAB24B10; red). In WT larvae, glia migrate appropriately to the lamina ganglia where they express the locorC56 marker. In WT and sgf11, glia correlate with mistargeting of photoreceptor axons.

(B-D) RNAi constructs against the indicated genes – (left and middle panels, green). Glial cells were labeled for comparison using anti-repo (middle and right panels, magenta). Maximum projection images of 0.5 μm z-stacks are shown for each knock-down. The positions of individual mistargeted photoreceptor axons are indicated by arrowheads in each panel. The expected position of lamina glial cells is indicated by dotted lines in the right panels for each genotype. Scale bars, 20 μm. RNAi, RNA interference; WT, wild-type.

An unexpected finding from this study was the overlap between genes that are transcriptionally regulated by SAGA deubiquitinase activity in glia and proteins that are sequestered by polyQ-ATXN7 into inclusion bodies in SCA7. These inclusion bodies that form in the nuclei of neuronal cells result from aggregation of the polyQ-mutant protein, and are a hallmark of polyQ diseases (Holmberg et al. 1998; Takahashi et al. 2003). Strikingly, several of the major classes of SAGA-regulated genes in glia, including those that regulate protein folding and protein degradation, also form the major protein components of these inclusion bodies. In particular, subunits of the 19S proteasome and the molecular chaperones are both transcriptionally regulated by SAGA in glia (this study) and found in SCA7-associated inclusions (Janer et al. 2010; Takahashi et al. 2002, 2003; Zander et al. 2001; Matilla et al. 2001). Notably, one of the most downregulated genes identified in SAGA deubiquitinase mutant glia, Hsp27, encodes a molecular chaperone that has reduced levels in SCA7 patients (Tsai et al. 2005) and that attenuates polyQ protein toxicity in a Drosophila model of neurodegenerative disease when overexpressed (Liao et al. 2008). The overlap between transcriptional targets of SAGA in glia and proteins that aggregate in SCA7 inclusion bodies suggests that SCA7 could reduce the levels of proteins involved in protein folding and proteasomal degradation in glial cells both directly and indirectly, through reduced SAGA deubiquitinase activity. It remains to be determined whether this further reduction in the levels of proteins that require SAGA deubiquitinase activity for expression in glia contributes to polyQ-toxicity and neurodegeneration in SCA7.

Previously, we showed that SAGA deubiquitinase activity was important for full expression of tissue-specific genes with developmental functions (Weake et al. 2011). When we compare the SAGA deubiquitinase-activated genes in embryonic muscle with those identified in glia in this study, we find that there are only 24 commonly downregulated genes. Since the genes that are activated by SAGA deubiquitinase activity differ so completely in these two different cell types, our findings indicate that the sensitivity of a particular gene to ubH2B-deubiquitination is dependent upon epigenetic factors rather than sequence information such as promoter motifs. Identifying the common chromatin landscape of genes that require SAGA deubiquitinase activity for expression may therefore provide insight into how tissue-specific gene expression is controlled at the chromatin level.

ACKNOWLEDGMENTS

Fly stocks from the Bloomington Drosophila Stock Center [National Institutes of Health (NIH) grant P400D018537], antibodies from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Developmental Studies Hybridoma Bank (funded by UL1TR001108), are gratefully acknowledged. RNA-seq data were collected using the genomics core facility supported by an NIH grant (P30 CA023168) to the Purdue University Center for Cancer Research. J.M. was supported by an Agricultural...
Research at Purdue Assistantship in Food and Agriculture from Purdue University. K.J.B. received funds from an American Society for Biochemistry and Molecular Biology Undergraduate Research Award. Support from the NIH (grant R01EY024905) to V.M.W. is gratefully acknowledged. The authors declare no competing financial interests.

**LITERATURE CITED**

Alfonso, T. B., and B. W. Jones, 2002 gcm2 promotes glial cell differentiation and is required with glial cells missing for macrophage development in Drosophila. Dev. Biol. 248(2): 369–383.

Anders, S., and W. Huber, 2010 Differential expression analysis for sequence count data. Genome Biol. 11(10): R106.

Bainton, R. J., L. T. Tsai, T. Schwabe, M. DeSalvo, U. Gaul et al., 2005 moody encodes two GPCRs that regulate cocaine behaviors and blood-brain barrier permeability in Drosophila. Cell 123(1): 145–156.

Bauke, A. C., S. Sasse, T. Matzat, and C. Klambt, 2015 A transcriptional network controlling glial development in the Drosophila visual system. Development 142(12): 2184–2193.

Bazigou, E., H. Apitz, J. Johansson, C. E. Loren, E. M. Hirst et al., 2008 Subtypes of glial cells in the Drosophila embryonic ventral nerve cord as related to lineage and gene expression. Mech. Dev. 125(5–6): 542–557.

Berger, J., K. A. Senti, G. Senti, T. P. Newsome, B. Asling et al., 2008 Systematic identification of genes that regulate neuronal wiring in the Drosophila visual system. PLoS Genet. 4(5): e1000085.

Bishop, J. R., M. R. Passos-Bueno, L. Fong, K. I. Stanford, J. C. Gonzales et al., 2010 Deletion of the basement membrane heparan sulfate proteoglycan type XVIII causes hypertrophicgicemia in mice and humans. PLoS One 5(11): e13919.

Bolger, A. M., M. Lohse, and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15): 2114–2120.

Bonnet, J. C., Y. Wang, T. Baptista, S. D. Vincent, W. C. Hsiao et al., 2014 The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes Dev. 28(18): 1999–2012.

Chang, T., A. Younossi-Hartenstein, and V. Hartenstein, 2003 Development of neural lineages derived from the sine oculis positive eye field of Drosophila. Arthropod Struct. Dev. 32(4): 303–317.

Chen, C. K., W. Y. Chen, and C. T. Chien, 2012 The POU-domain protein Lobsiger, C. S., and D. W. Cleveland, 2007 Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat. Neurosci. 10(11): 1355–1360.

Edwards, T. N., and I. A. Meinertzhagen, 2010 The functional organisation of glia in the adult brain of Drosophila and other insects. Prog. Neurobiol. 90(4): 471–497.

Enevoldson, T. P., M. D. Sanders, and A. E. Harding, 1994 Autosomal dominant cerebellar ataxia with pigmentary macular dystrophy. A clinical and genetic study of eight families. Brain 117(Pt 3): 445–460.

Edgar, R., M. Domrachev, and A. E. Lash, 2002 Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30(1): 207–210.

Edwards, T. N., and I. A. Meinertzhagen, 2010 The functional organisation of glia in the adult brain of Drosophila and other insects. Prog. Neurobiol. 90(4): 471–497.

Enevoldson, T. P., M. D. Sanders, and A. E. Harding, 1994 Autosomal dominant cerebellar ataxia with pigmentary macular dystrophy. A clinical and genetic study of eight families. Brain 117(Pt 3): 445–460.

Fischer, J. A., A. Acosta, A. Kenny, C. Cather, C. Robinson et al., 2004 Drosophila karskis has distinct subcellular localization domains for nuclear envelope and microtubule localization in the eye. Genetics 168(3): 1385–1393.

Freeman, M. R., J. Delrow, J. Kim, E. Johnson, and C. Q. Doe, 2003 Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. Neuron 38(4): 567–580.

Fujita, S. C., S. L. Zipursky, S. Benzer, A. Ferrus, and S. L. Shotwell, 1982 Monoclonal antibodies against the Drosophila nervous system. Proc. Natl. Acad. Sci. USA 79(24): 7929–7933.

Garrity, P. A., Y. Rao, I. Salecker, J. McClade, T. Pawson et al., 1996 Drosophila photoreceptor axon guidance and targeting requires the dreadlocks SH2/SH3 adapter protein. Cell 85(5): 639–650.

Granderath, S., A. Stollwerck, S. Greig, C. S. Goodman, C. J. O’Kane et al., 1999 loco encodes an RGS protein required for Drosophila glial differentiation. Development 126(8): 1781–1791.

Halter, D. A., J. Urban, C. Rickert, S. S. Ner, K. Ito et al., 1995 The homeobox gene repo is required for the differentiation and maintenance of glia function in the embryonic nervous system of Drosophila melanogaster. Development 121(2): 317–332.

Heljasaava, R., P. Nyberg, J. Luostarinen, M. Parikka, P. Heikkila et al., 2005 Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteinases. Exp. Cell Res. 307(2): 292–304.

Henry, K. W., A. Wyce, W. S. Lo, L. I. Duggan, N. C. Emre et al., 2003 Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev. 17(21): 2648–2663.

Holmberg, M., C. Duyckaerts, A. Durr, G. Cancel, I. Gourfinkel-An et al., 1998 Spinecerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. Hum. Mol. Genet. 7(5): 913–918.

Huang, Z., and S. Kunes, 1996 Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the Drosophila brain. Cell 86(3): 411–422.

Janker, A., A. Werner, J. Takahashi-Fujigasaki, A. Daret, H. Fujigasaki et al., 2010 SUMOylation attenuates the aggregation propensity and cellular toxicity of the polyglutamine expanded ataxin-7. Hum. Mol. Genet. 19(1): 181–195.

Kohler, A., E. Zimmerman, M. Schneider, E. Hurt, and N. Zheng, 2010 Structural basis for assembly and activation of the heterotrimeric SAGA histone H2B deubiquitination module. Cell 141(4): 606–617.

Lan, X., E. Koutelou, A. C. Schiller, Y. C. Chen, P. A. Grant et al., 2015 Poly(Q) Expansions in ATXN7 Affect Solubility but Not Activity of the SAGA Deubiquitinating Module. Mol. Cell. Biol. 35(10): 1777–1787.

Langmead, B., and S. L. Salzberg, 2012 Fast gapped-read alignment with Bowtie 2. Nat. Methods 9(4): 357–359.

Li, Y., and R. W. Padgett, 2012 bantam is required for optic lobe development and glial cell proliferation. PLoS One 7(3): e32910.

Liao, P. C., H. Y. Lin, C. H. Yuh, L. K. Yu, and H. D. Wang, 2008 The effect of neuronal expression of heat shock proteins 26 and 27 on lifespan, neurodegeneration, and apoptosis in Drosophila. Biochem. Biophys. Res. Commun. 376(4): 637–641.

Lobsiger, C. S., and D. W. Cleveland, 2007 Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat. Neurosci. 10(11): 1355–1360.

Love, M. I., W. Huber, and S. Anders, 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15(12): 550.

Ma, J., and V. M. Weake, 2014 Affinity-based isolation of tagged nuclei from Drosophila tissues for gene expression analysis. J. Vis. Exp. 85: e51418.

Madsen, J. G., S. F. Schmidt, B. D. Larsen, A. Loft, R. Nielsen et al., 2015 RNA-seq: computational method for genome-wide assessment of acute transcriptional regulation from total RNA-seq data. Nucleic Acids Res. 43(6): e40.

Mardon, G., N. M. Solomon, and G. M. Rubin, 1994 dachshund encodes a nuclear protein required for normal eye and leg development in Drosophila. Development 120(12): 3473–3486.
Martin, K. A., B. Poecck, H. Roth, A. J. Ebens, L. C. Ballard et al., 1995 Mutations disrupting neuronal connectivity in the Drosophila visual system. Neuron 14(2): 229–240.

Martin-Morris, L. E., and K. White, 1990 The Drosophila transcript encoded by the beta-amyloid protein precursor-like gene is restricted to the nervous system. Development 110(1): 185–195.

Matilla, A., C. Gorbea, D. B. Einum, J. Townsend, A. Michalk et al., 2001 Association of axatin-7 with the proteasome subunit S4 of the 19S regulatory complex. Hum. Mol. Genet. 10(24): 2821–2831.

Mayer, F., N. Mayer, L. Chinn, R. L. Pinsonneault, D. Kroetz et al., 2009 Evolutionary conservation of vertebrate blood-brain barrier chemoprotective mechanisms in Drosophila. J. Neurosci. 29(11): 3558–3550.

McCullough, S. D., X. Xu, S. Y. Dent, S. Bekiranov, R. G. Roeder et al., 2012 Reelin is a target of polyglutamine expanded axatin-7 in human spinocerebellar ataxia type 7 (SCA7) astrocytes. Proc. Natl. Acad. Sci. USA 109(52): 21319–21324.

Meyer, F., and B. Moussian, 2009 Drosophila multiplexin (Dmp) modulates motor axon pathfinding accuracy. Dev. Growth Differ. 51(5): 483–498.

Paisan-Ruiz, C., G. Scopes, P. Lee, and H. Houlden, 2009 Homozygosity mapping through whole genome analysis identifies a COL18A1 mutation in an Indian family presenting with an autosomal recessive neurological disorder. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 150B(7): 997–993.

Pappu, K. S., M. Morey, A. Nern, B. Spitzweck, B. J. Dickinson et al., 2011 Robo-3–mediated repulsive interactions guide R8 axons during Drosophila visual system development. Proc. Natl. Acad. Sci. USA 108(18): 7571–7576.

Patterson, K., A. B. Molofsky, C. Robinson, S. Acosta, C. Cater et al., 2004 The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the Drosophila eye. Mol. Biol. Cell 15(2): 600–610.

Pecot, M. Y., Y. Chen, O. Akin, Z. Chen, C. Y. Tsui et al., 2014 Sequential axon-derived signals couple target survival and layer specificity in the Drosophila visual system. Neuron 82(2): 320–333.

Rabinowitz, W., D. Shy, and V. Hartenstein, 2005 Morphogenesis and proliferation of the larval brain glia in Drosophila. Dev. Biol. 283(1): 191–203.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth, 2010 edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26(1): 139–140.

Sepp, K. J., J. Schulte, and V. J. Auld, 2001 Peripheral glia direct axon guidance across the CNS/PNS transition zone. Dev. Biol. 238(1): 47–63.

Sertie, A. L., V. Sossi, A. A. Camargo, M. Zatz, C. Brabe et al., 2000 Collagen XVIII, containing an endogenous inhibitor of angiogenesis and tumor growth, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome). Hum. Mol. Genet. 9(13): 2051–2058.

Step, S. E., H. W. Lim, J. M. Marinis, A. Prokesch, D. J. Steger et al., 2014 Anti-diabetic rosiglitazone remodels the adipocyte transcriptome by redistributing transcription to PPARgamma-driven enhancers. Genes Dev. 28(9): 1018–1028.

Sweeney, S. T., A. Hidalgo, J. S. de Belle, and H. Keshishian, 2012 X-gal staining of the central nervous system in adult Drosophila. Cold Spring Harb. Protoc. 2012(2): 239–241.

Takahashi, J., H. Fujigasaki, C. Zander, K. H. El Hachimi, G. Stevanin et al., 2002 Two populations of neuronal intranuclear inclusions in SCA7 differ in size and promyelocytic leukaemia protein content. Brain 125(Pt 7): 1534–1543.

Takahashi, J., H. Fujigasaki, K. Iwabuchi, A. C. Bruni, T. Uchihara et al., 2003 PML nuclear bodies and neuronal intranuclear inclusion in polyglutamine diseases. Neurobiol. Dis. 13(3): 230–237.

Thomas, G. R., and D. J. van Meyel, 2007 The glycosyltransferase Fringe promotes Delta-Notch signaling between neurons and glia, and is required for subtype-specific glial gene expression. Development 134(3): 591–600.

Timofeev, K., W. Joly, D. Hadjieconomou, and I. Salecker, 2012 Localized netrins act as positional cues to control layer-specific targeting of photoreceptor axons in Drosophila. Neuron 75(1): 80–93.

Trappell, C., L. Pachter, and S. L. Salzberg, 2009 TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25(9): 1105–1111.

Tsai, H. F., S. J. Lin, C. L., and M. Hsieh, 2005 Decreased expression of Hsp27 and Hsp70 in transformed lymphoblastoid cells from patients with spinocerebellar ataxia type 7. Biochem. Biophys. Res. Commun. 334(4): 1279–1286.

Umetu, D., S. Murakami, M. Sato, and T. Tabata, 2006 The highly ordered assembly of retinal axons and their synaptic partners is regulated by Hedgehog/Single-minded in the Drosophila visual system. Development 133(5): 791–800.

Wang, T., A. G. Hausrath, A. Tong, D. K. Dickman, and G. W. Davis, 2014 Endostatin is a trans-synaptic signal for homoeostatic synaptic plasticity. Neuron 83(3): 616–629.

Weake, V. M., K. K. Lee, S. Guelman, C. H. Lin, C. Seidel et al., 2008 SAGA-mediated H2B deubiquitination controls the development of neuronal connectivity in the Drosophila visual system. EMBO J. 27(2): 394–405.

Weng, M., K. L. Golden, and C. Y. Lee, 2010 dFzd/Earnulf maintains the restricted developmental potential of intermediate neural progenitors in Drosophila. Dev. Cell 18(1): 126–135.

Wickstrom, S. A., K. Alitalo, and J. Keskı-Ojja, 2005 Endostatin signaling and regulation of endothelial cell-matrix interactions. Adv. Cancer Res. 94: 197–229.

Winberg, M. L., S. E. Perez, and H. Steller, 1992 Generation and early differentiation of glial cells in the first optic ganglion of Drosophila melanogaster. Development 115(4): 903–911.

Xiong, W. C., H. Okano, N. H. Patel, J. A. Blendy, and C. Montell, 1994 repo encodes a glial-specific homeo domain protein required in the Drosophila nervous system. Genes Dev. 8(8): 981–994.

Yang, H., S. Liu, W. T. He, J. Zhao, L. L. Jiang et al., 2015 Aggregation of Polyglutamine-expanded Ataxin 7 Protein Specifically Sequesters Ubiquitin-specific Protease 22 and Deteriorates Its Deubiquitinating Function in the Spt-Ada-Gcn5-Acetyltransferase (SAGA) Complex. J. Biol. Chem. 290(36): 21996–22004.

Yu, J., D. A. Starr, X. Wu, S. M. Parkhurst, Y. Zhuang et al., 2006 The KASH domain protein MSP-300 plays an essential role in nuclear anchoring during Drosophila oogenesis. Dev. Biol. 289(2): 336–345.

Yuvu-Aydemir, Y., A. C. Buke, and C. Klambt, 2011 Spinster controls Dpp signaling during glial migration in the Drosophila eye. J. Neurosci. 31(19): 7005–7015.

Zander, C., J. Takahashi, K. H. El Hachimi, H. Fujigasaki, V. Albanese et al., 2001 Similarities between spinocerebellar ataxia type 7 (SCA7) cell models and human brain: proteins recruited in inclusions and activation of caspase-3. Hum. Mol. Genet. 10(22): 2569–2579.

Zhang, X. Y., M. Varthi, S. M. Sykes, C. Phillips, C. Warzeka et al., 2008 The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol. Cell 29(1): 102–111.

Zhao, Y., G. Lang, S. Ito, J. Bonnet, E. Metzger et al., 2008 A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol. Cell 29(1): 92–101.

Zhu, C. C., J. Q. Boone, P. A. Jensen, S. Hanna, L. Podemski et al., 2008 Drosophila Activin- and the Activin-like product Dawdle function redundantly to regulate proliferation in the larval brain. Development 135(3): 513–521.

Communicating editor: H. K. Salz