Appl-GAL4 driven transcription in adult heads

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Drosophila melanogaster is one of the most important invertebrate model organisms to study the aging process. The proper application of genetic tools that depend on the GAL4/UAS bipartite gene expression system to overexpress or downregulate genes of interest in aging research requires GAL4 drivers that provide well characterized expression levels in specific adult tissues. It was previously reported, that Appl-GAL4 driven expression of UAS-Atg8a lines increased maximal and average lifespan, while the widely used pan-neuronal elav-GAL4 driver did not provide similar effect (Simonsen, 2008), suggesting that Appl-GAL4 provides stable adult expression. To assess the expression level and exression pattern of Appl-GAL4 in the adult nervous system we decided to characterize UAS transcription driven by the P{w[+m*]=Appl-GAL4.G1a}1, y[1] w[*] line (BDSC stock no. 32040) that is advertised to expresses GAL4 in the nervous system.

In order to determine UAS transgene expression levels driven by Appl-GAL4 during aging we collected freshly eclosed *Appl-GAL4/w; UAS-GFP/+* progeny of *Appl-GAL4* females and *w; UAS-GFP* males and maintained them at 25 °C by passing them to fresh vials every 2-3 days. We prepared RNA samples from heads of 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks old female flies (10 heads per sample, three biological replicates per age group) using QIAzol Lysis Reagent (Qiagen). We generated first strand cDNA from 0.5 µg RNA per sample using TaqMan Reverse Transcription Reagents (Thermo Scientific) with random hexamer primers, then measured transgene expression levels in a PikoReal Real-Time PCR system (Thermo Scientific) using GoTaq qPCR Master Mix (Promega) with primers specific for pUAST (Fw: CTG TGG TGT GAC ATA ATT GGA CAA, Rev: TGC TCC CAT TCA TCA GTT CC A, designed for the SV40 polyA/terminator region in the pUAST vector) and with primers for the rp49 housekeeping gene that was used for normalization. qPCRs were performed in duplicates and transcript levels were determined by setting Ct values against cDNA template calibration curves. We found that the transcript level of UAS-GFP significantly decreased over time (Spearman's

correlation coefficient ρ=-0.60553, P=0.00363). A gradual reduction in Appl-GAL4 driven UAS-GFP expression was most pronounced after 2 weeks of age, with heads of 4 and 6 weeks old flies having transcript levels below 60% of that of freshly eclosed adults (Fig. 1).

Next, we investigated the expression pattern of Appl-GAL4 in the adult nervous system by visualizing GFP expression in dissected brains of 1 week old *Appl-GAL4/w; UAS-GFP/+*females under a Nikon Eclipse 80i fluorescent microscope (Fig. 2). We found that the adult neuronal expression driven by Appl-GAL4 is not uniform. Although low levels of GFP could be detected in most parts of the brain, high level expression was detected only in the mushroom body.

In conclusion, we found that Appl-GAL4 driven UAS expression is not uniform in the adult brain and its level decreases during aging - both issues should be taken into consideration in aging studies.

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References

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Figure legends

Figure 1: Expression level of Appl-GAL4 driven UAS-GFP in heads of aging adults. Bars show the average of three biological replicates, error bars represent the standard error of mean.

Figure 2: Appl-GAL4 driven expression of UAS-GFP in the adult brain.

Figure 1.

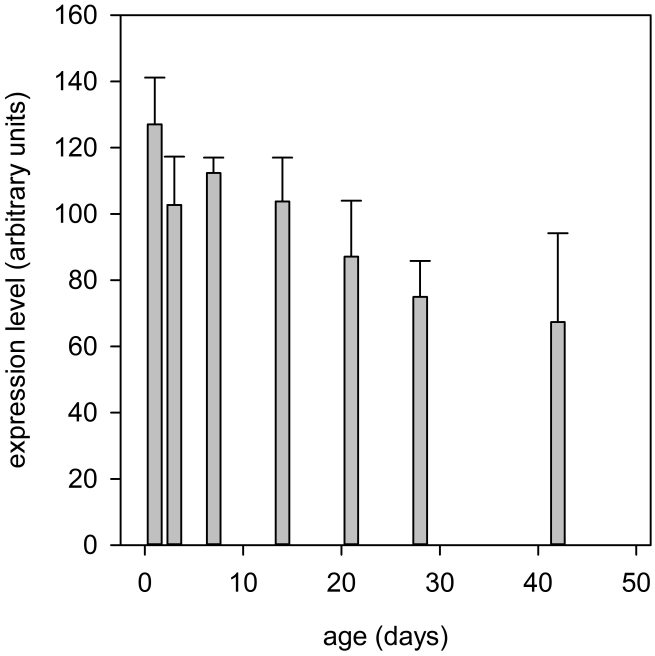

Figure 2.