**Split-QF System for Fine-Tuned Transgene Expression in Drosophila**

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**ABSTRACT** The Q-system is a binary expression system that works well across species. Here, we report the development and demonstrate the applications of a split-QF system that drives strong expression in *Drosophila*, is repressible by QS, and is inducible by a small nontoxic molecule (quinic acid). The split-QF system is fully compatible with existing split-GAL4 and split-LexA lines, thus greatly expanding the range of possible advanced intersectional experiments and anatomical, physiological, and behavioral assays in *Drosophila*, and in other organisms.

**KEYWORDS** Q-system; quinic acid; split-GAL4; split-LexA

**Materials and Methods**

**Molecular biology**

Plasmids were constructed by standard procedures including enzyme digestions, PCR, and subcloning, using the In-Fusion HD Cloning System CE (Takara Bio Europe # 639636). Plasmid inserts were verified by DNA sequencing.

**nsyb-nls::QFAD::Zip+ construct:**

1. The pattB-QF2-hsp70 plasmid (#46115; Addgene) was digested with ZraI and EcoRI to remove the Kozak-QF2 sequence.

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2. The Kozak-nls sequence was PCR-amplified from pBPp65ADZpUw (#26234; Addgene) with primers 5'-ATC GAC AGC CGA ATT CAA TTA GAA AGC GGA ATT A-3' (forward) and 5'-AGG GTA TCG ATA GAC GTC CAA TCC GAC TCT TCT CTT C-3' (reverse).

3. The PCR product was cloned into the digested vector by InFusion cloning.

4. The cloning product was digested with ZraI.

5. The GSOS-GSOS sequence was PCR-amplified from the pattB-QF2-hsp70 plasmid (#46115; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

6. The PCR product from step 5 was subcloned into the cloning product from step 4 by InFusion cloning.

7. The product from step 6 was digested with BglII.

8. The GSOS-GSOS sequence was PCR-amplified from pBPp65ADZpUw (#26234; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

9. The PCR product from step 8 was subcloned into the digested vector from step 7 by InFusion cloning.

10. The product from step 9 was digested with FseI and NotI to remove the hsp70 terminator, and to replace it with the simian virus 40 (SV40) terminator.

11. The SV40 terminator was PCR-amplified from the UAS-LUC-UAS-eYFP plasmid (Lynd and Lycett 2012) with primers 5'-GGG GCC TAA GGG CCG CCG ATC TCT GTG AAG GAA CAA 'TAC-3' (forward) and 5'-CGT CGA GCC GCG GCC GCC ATC CAG ACA TGA TAA GAT AC-3' (reverse).

12. The PCR product from step 11 was subcloned into the vector from step 10 by InFusion cloning.

**nsyb-nls::QF2*AD::Zip+ construct:**

1. The nsyb-nls::QFAD::Zip+ construct was digested with BglII and ZraI to remove QFAD.

2. The QF2*AD sequence was PCR-amplified from pBPp65ADZpUw (#26234; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

3. The PCR product from step 2 was cloned into the product from step 1 by InFusion cloning.

**nsyb-nls::p65AD::Zip+ construct:**

1. The nsyb-nls::QFAD::Zip+ construct was digested with FseI and ZraI to remove the QFAD::Zip+ sequence.

2. The p65AD::Zip+ sequence was PCR-amplified from pBPp65ADZpUw (#26234; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

3. The PCR product from step 2 was cloned into the product from step 1 by InFusion cloning.

**nsyb-nls::GAL4AD::Zip+ construct:**

1. The nsyb-nls::QFAD::Zip+ construct was digested with BglII and ZraI to remove QFAD.

2. The GAL4AD sequence was PCR-amplified from pBPGA-L4.2Uw-2 (#26227; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

3. The PCR product from step 2 was cloned into the product from step 1 by InFusion cloning.

**nsyb-Zip--::QFDBD construct:**

1. The pattB-QF2-hsp70 plasmid (#46115; Addgene) was digested with ZraI and EcoRI to remove the Kozak-QF2 sequence.

2. The Kozak-Zip--GGGGGG sequence was PCR-amplified from pBPpZpGAL4DBDUw (#26233; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

3. The PCR product from step 2 was InFusion-subcloned into the product from step 1.

4. The product from step 3 was digested with ZraI.

5. QFDBD was PCR-amplified from pBPpZpGAL4DBDUw (#46115; Addgene) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

6. The PCR product from step 5 was InFusion-subcloned into the product from step 4.

7. The product from step 6 was digested with FseI and NotI to remove the hsp70 terminator and replace it with the simian virus 40 (SV40) terminator.

8. The SV40 terminator was PCR-amplified from the UAS-LUC-UAS-eYFP plasmid (Lynd and Lycett 2012) with primers 5'-ATA CGA ACA GAG ATC TGG AGG AGG TGG TGG AGG-3' (forward) and 5'-ATC GAT AGA CAG ATC GGC CCG CCT 'TAC TGT CGG CCG CC-3' (reverse).

9. The PCR product from step 8 was InFusion-subcloned into the product from step 7.

**New and existing transgenic flies**

New transgenic lines were generated by inserting the nsyb-QFDBD construct in attp40 (II) and all nsyb-AD constructs into attp2 (III). New stocks were deposited to the Bloomington *Drosophila* Stock Centre as ##81281 - 81302.

Other *Drosophila* stocks used in this paper were acquired from the Bloomington *Drosophila* Stock Centre (indicated by # below) or were in personal stocks of the authors. Figure 1: QUAS-mCD8-GFP (#30003), tub-QS (#52112), nsyb-QF2 (attp2, personal stocks, O.R.), nsyb-QF2 (##81281 - 81302), QUAS-Ppyr/Luc (#64773); Figure 2: UAS-mCD8-GFP
LuciferaseMeasurement

To take images that were compared to each other. Images shown before experimental images. Identical settings were used to automatically by taking an image of a white sheet of paper source, and LAS v.4 software. The white balance was adjusted with a Leica DFC 420C camera, QImaging LED light taken on a Leica MZ10F zoom fluorescence microscope equipped with an HCX IRAP0 1.25x/0.95W water-immersion objective (S06323), at 512 x 512 pixel resolution with 1 μm z steps. LAS X v.3.5.2 software was used for image acquisition. Imaging settings (laser intensity, gain, etc.) were kept identical for groups of images that were compared to one another. Images were processed by taking the maximum intensity projection, rotating, and recoloring in Fiji. Images shown are representative of three-to-five staining experiments for every genotype.

Whole-animal imaging

Third-instar larvae were placed on a microscope slide and briefly put into a freezer to immobilize them. Images were taken on a Leica MZ10F zoom fluorescence microscope equipped with a Leica DFC 420C camera, QImaging LED light source, and LAS v4 software. The white balance was adjusted automatically by taking an image of a white sheet of paper before experimental images. Identical settings were used to take images that were compared to each other. Images shown are representative of three to five experiments for every genotype.

QA feeding

For larval experiments, gravid females were allowed to lay eggs in vials containing standard fly medium, supplemented with QA, and larvae remained in the vials until they reached the wall-climbing third-instar stage. For adult experiments, flies were raised on standard fly medium and were transferred into vials with QA at 2–3 d.o.f. for 5 days, at which point they were dissected. To make QA stock, 8 g of QA (#138622; Sigma [Sigma Chemical], St. Louis, MO) was dissolved in 40 ml ddH2O and adjusted to pH 7 with 5 M NaOH, bringing the total stock volume to 50 ml. Next, 1.6 ml/vial of this solution was thoroughly mixed into standard fly medium for larval or adult experiments.

Luciferase assay

Each experiment assayed 9–30 larvae or 9–15 adult flies per genotype in groups of three. Third-instar larvae or 1–2 d.o. adult flies were placed in a 1.5 ml Eppendorf tube and stored at −80°C until all samples for a given experiment were collected. A Dual-Luciferase Reporter Assay system (E1910; Promega, Madison, WI) was used for the experiments. Samples were homogenized in 200 μl of passive lysis buffer (E194A; Promega) per tube and kept on ice for ≥10 min. Then, the tubes were centrifuged for 5 min at 13,400 rpm and the supernatants transferred to new tubes. Next, 30 μl of supernatant from each tube were mixed with 30 μl of luciferase assay substrate (E151A; Promega), reconstituted in luciferase assay buffer (E195A; Promega) per well of a 96-well plate, and luminescence was measured immediately on a TECAN GENios plate reader, running XFluor 4 macros for Excel. We used 300-msec exposure for adult samples and 600 msec exposure for larval samples. We collected 3–10 measurements per experiment per genotype. The luciferase luminescence values were normalized by the amount of protein contained in the samples, to account for possible differences in the sizes of larvae and adults. For protein assay, 1.5 μl of supernatant was mixed with 100 μl of protein assay reagent (#500-0006; Bio-Rad, Hercules, CA) and light absorbance measured after 20 min on a FLUOstar Omega plate reader (BMG LABTECH) running Omega software v.1.3. Two independent samples were measured per supernatant tube. The absorbance values were converted into milligrams per milliliter of protein by measuring a calibration curve with BSA dilutions (#B90015; New England Biolabs, Beverly, MA). All relative luminescence (RL) data points presented on the graphs (Figure 1, C and D, Figure 2, B and C, and Figure 3, A and B) were calculated as follows:

\[
RL = \frac{\text{LuciferaseMeasurement}}{\text{DerivedProtein}}
\]

where

\[
\text{DerivedProtein} = 30 \left( a \left( \frac{\text{ProteinMeasurement}_1 + \text{ProteinMeasurement}_2}{2} \right) - \text{BlankMeasurement} \right) + b
\]
\(a\) and \(b\) parameters were obtained from the best linear fit to the calibration curve, plotted as \([\text{average of three calibration measurement for a given dilution of BSA]} - \text{blank measurement}] \text{vs. [dilution of BSA in milligrams per milliliter]}\). Per genotype, four to six independent RL values were collected in each experiment. The genotypes are presented in the figures as mean ± SEM, and were compared with one- (larvae) or two- (adults) way ANOVA with Sidak’s multiple comparisons test.

We have observed significant differences between the measurements of adult males and females for some genotypes,
arising from a consistently higher amount of protein per adult female. These differences were never observed for male and female larvae (data not shown). Thus, we present adult data separately for males and females.

**Larval whole-cell patch-clamp recordings**

Larvae were grown in the dark on standard fly medium, supplemented with 100 μl/vial of 0.1 M all trans-retinal (#R2500; Sigma) in 100% EtOH. Recordings were performed at RT (20–22°C). Third-instar larvae were dissected in external saline (135 mM NaCl, 5 mM KCl, 4 mM MgCl2·6H2O, 2 mM CaCl2·2H2O, 5 mM N-Tris(hydroxymethyl)methyl-2-aminothanesulfonic acid, and 36 mM sucrose, pH 7.15). For each larva, the CNS was removed and secured to a Sylgard-coated (Dow-Corning, Midland, MI) cover slip using tissue glue (Glutamate; WP, Hitchin, UK). The glia surrounding the CNS were partially removed using protease (1% type XIV; Sigma) contained in a wide-bore (15 μm) patch pipette. Whole-cell recordings were carried out using borosilicate glass electrodes (GC100TF-10; Harvard Apparatus, Edenbridge, UK), fire-polished to resistances of between 10 and 14 MΩ. The aCC/RP2 motoneurons were identified by soma position within the ventral nerve cord. When needed, cell identity was confirmed after recording by filling with 0.1% Alexa Fluor 488 hydrazide sodium salt (Invitrogen), included in the internal patch saline (140 mM potassium gluconate, 2 mM MgCl2·6H2O, 2 mM EGTA, 5 mM KCl, and 20 mM HEPES, pH 7.4). Mecamylamine (1 mM, M9020; Sigma) was included in the external saline to block endogenous excitatory cholinergic-mediated currents to aCC/RP2 motoneurons and neuronal depolarization was elicited through UAS-ChR2 (Pulver et al. 2009) (λ470 nm, 500 msec, light intensity 9.65 mW/cm2 before reaching the LUMPlanFI 60x/0.9W Olympus objective) expressed in all motoneurons by the VGlut promoter. Recordings were made using a MultiClamp 700B amplifier. Cells were held at −55 mV, and recordings were sampled at 20 kHz and low-pass filtered at 10 kHz using pClamp 10.6 (Molecular Devices, Sunnyvale, CA). Only neurons with an input resistance of ≥ 500 MΩ were accepted for analysis. Eight recordings were taken per cell, average action potential number was calculated per 500 msec light pulse. Data in Figure 3E are presented as mean ± SEM, and were compared with one-way ANOVA with Sidak’s multiple comparisons test.

**Larval escape assays**

Individual third-instar larvae were assayed at RT (20–22°C) in a 9-cm Petri dish that contained a thin layer of 1% agarose to prevent desiccation. The Petri dish was placed under a Leica MZ16F zoom fluorescence microscope with a Plan 1.0× lens, fluorescence light source, and a GFP filter cube (λ470 nm). Light intensity measured 9.87 mW/cm² when completely zoomed out. Zoom 5 was used for experiments. Larvae were filmed using a uEye UI-233xSE-C camera with uEye Cockpit software, and data were stored in *.avi format. Each larva was allowed to crawl in the Petri dish for 2 min, before it was placed for 2 min in a 113-mm² area illuminated by blue light. Wild-type larvae naturally avoid bright blue light and crawl away; however, larvae with ChR2 expressed in motoneurons (Figure 3F) or pan-neuronally (Figure 3G) are impaired in their ability to escape. A larva was returned into the blue-light area immediately after it had completely left the illuminated area. We counted the number of escapes during a 2-min period. Per genotype, 7–15 larvae were assayed. The data are shown as mean ± SEM. The genotypes were compared with one-way ANOVA with Sidak’s multiple comparisons test.

**Adult behavioral assay**

Adult male and female 5–7 d.o. flies were assayed in groups of 10 (N = 4–5 groups per genotype) in clean empty standard fly vials. Flies were placed in a cooled incubator set to 33°C, and video-recorded at 5 frames per second using a uEye camera UI-233xSE-C, controlled by uEye Cockpit software. The data were stored in *.avi format. The number of flies on the bottom of each vial was manually counted at 30-sec intervals. The data are shown as mean ± SEM, and were analyzed with multiple Student’s t-tests with Holm–Sidak correction.

**Data availability**

Fly strains generated in this study are available from the Bloomington Drosophila Stock Centre and upon request from the corresponding author. Plasmids generated in this study are available upon request from the corresponding author. Supplemental material available at https://doi.org/10.25386/genetics.7801160.

**Results**

**Quantification of strength of split-QF transactivators**

To make the split-QF system compatible with existing split-GAL4 lines, we used the same leucine zippers (Pfeiffer et al. 2010). We attached Zip− to QFDBD and Zip+ to QFAD, defining the domains as previously reported (Ribabinina et al. 2015), and expressed these transgenes under control of the neuronal synaptobrevin promoter nsyb (Figure 1A), integrating them in attp40 (DBD) and attp2 (AD) sites. We also generated QF2“AD::Zip+ flies (in attp2) that, similar to QF2“ transactivator (Ribabinina et al. 2015) (Figure 1A), carry a mutated C-terminal with reduced negative charge and reduced activity. As expected, animals carrying nsyb-QFDBD (attp40), nsyb-QFAD (attp2), and QUAS-mCD8-GFP showed strong GFP expression throughout their nervous system (Figure 1B). This expression was repressible by tub-QS and inducible by QA (Supplemental Material, Figure S1). Similar, but weaker, expression was observed with nsyb-QFDBD and nsyb-QF2“AD (Figure 1B). Both split transactivators appeared to have lower activity than QF2 and QF2“ (Figure 1B).

To quantify the relative strength of split transactivators, and to compare QFAD and QF2“AD to existing p65AD and
tub-QS activator was 2.2 times (data not shown). The strength of the QFDBD+QFAD transgenotypes. No such differences were observed for larvae (Figure 1, C and D, and Tables S1 and S2). We analyzed male and female flies separately due to the significant differences between sexes that were observed for some genotypes. No such differences were observed for larvae. The strength of the QFDBD+QFAD transactivator was 2.2 times ($P < 0.0001$) lower than QF2 in larvae and 1.8–2 times ($P < 0.0001$) lower than QF2 in adults. Similarly, QFDBD+QF2wAD was three times ($P < 0.0001$) weaker than QF2w in larvae and 1.4–2.6 times weaker in adults ($P = 0.19$ in females and $P < 0.0001$ in males). While relative expression levels varied between larval (nonsexed) and male vs. female adults, QFAD was almost two times ($P < 0.01$) stronger than QF2wAD, and almost two times ($P < 0.0001$) weaker than p65AD. The GAL4AD was consistently weak. tub-QS provided strong repression of all original and split QF variants. We quantified the effect of QA derepression in larvae only, because in the adult brain QA is effective only in sensory receptor neurons and the pars intercerebralis neurons (Riabinina et al. 2015), presumably due to difficulty crossing the glial blood–brain barrier (Edwards and Meinertzhagen 2010). QA feeding to tub-QS, nsyb-QFDBD, nsyb-QFAd (QF2wAD) larvae, which otherwise had very low expression, resulted in restoration of expression to levels not significantly different from those of nsyb-QFDBD, nsyb-QFAd (QF2wAD) larvae ($P = 0.87$ and $P = 0.62$, respectively). In tub-QS, nsyb-QF2 (QF2w) larvae, the expression was restored to 50–60% of unrepressed levels ($P < 0.0001$ and $P = 0.0031$, respectively). These experiments demonstrate that the split-QF is fully functional, repressible, and inducible, due to the strong activity of the QFAD and QF2wAD activation domains.

Quantification of split-QF transactivators when used with split-GAL4 and split-LexA

Next, we asked whether QFAD and QF2wAD may be effectively used together with existing GAL4DBD lines to provide a QS-repressible and QA-inducible alternative to the currently used p65AD. Pan-neuronal expression in the larval CNS, driven by elav-GAL4DBD and nsyb-QF2/QF2wAD, is strong, repressible, and inducible (Figure 2A, top). To investigate expression in the adult brain, we used GAL4DBD lines from the Janelia and Vienna collections, in combination with nsyb-QFAD and nsyb-QF2wAD, to drive GFP expression in antennal lobes, suboesophageal zone (SEZ), antennal mechanosensory and motor centers, optic lobes, and sparsely in other areas of the brain (Figure 2A, bottom and Figure S2). The observed expression was strong and repressible in all neurons in the predicted expression patterns, and QA-inducible in the olfactory and gustatory receptor neurons, as observed previously with nsyb-QF2 (Riabinina et al. 2015). To quantify the strength of expression, we used elav-GAL4DBD in combination with the AD variants to drive luciferase in the CNS of third-instar larvae; however, the elav-GAL4DBD, nsyb-p65AD combination was lethal (Figure 2B and Table S3). Similarly to experiments in Figure 1C, QFAD-induced expression was not significantly different from QF2wAD ($P = 0.16$). In contrast to experiments with split-QF (Figure 1C), here, QA resulted in restoration of expression to ~20–35% of that of the unrepressed transactivators ($P < 0.0001$). Similarly to QF2 and QF2w, QA feeding restored expression levels of tub-QS, nsyb-GAL4QF to 60% of the unrepressed levels ($P < 0.0001$). To quantify expression levels in the adult CNS, we used ChAT-GAL4DBD to target cholinergic neurons and to avoid larval lethality, previously observed with elav-GAL4DBD, nsyb-p65AD (Figure 2C and Table S4). QFAD-driven expression was comparable with QF2wAD ($P > 0.99$) and almost four times weaker than p65AD ($P < 0.0001$). As previously observed (e.g., Figure 1, C and D), tub-QS provided strong repression that did not differ from DBD- or AD-only controls ($P > 0.99$). These experiments demonstrate that QFAD and QF2wAD activation domains may be used together with GAL4DBD lines to provide a repressible and inducible, albeit weaker, alternative to p65AD.

The QFAD and QF2wAD activation domains also work with split-LexA reagents in the larval and adult CNS (Figure 2D and Figure S3). Moreover, expression is again both repressible and QA-inducible. Although we did not quantify the strength of expression by luciferase assay (due to the unavailability of a LexAop-Luc reporter), it appears that the QF2wAD domain works as well, or better, than QFAD in these experiments.

Applications of split-QF

First, we asked how the QS repression compares with Killer-Zipper (Dolan et al. 2017), a tool that silences split-GAL4 expression by driving GAL4DBD-Zip+ construct with the LexA/LexAop system (Figure 3, A and B and Table S5). We observed that QS-induced repression was stronger than ($P = 0.0071$ for nsyb-QFDBD, nsyb-QFAD, KZip vs. tub-QS, nsyb-QFDBD, nsyb-QFAD females) or the same (all other genotypes, $P > 0.83$) as a Killer-Zipper-induced equivalent. The use of QS for repression is thus more advantageous than Killer-Zipper because it requires fewer transgenes and does not recruit the LexA/LexAop system. In addition, the mechanism of QS repression is different from that of Killer-Zipper, which is based on competitive dimerization between GAL4DBD-Zip+ and GAL4DBD-Zip− components.

Next, we tested whether the split-QF system may be effectively used for simultaneous expression of UAS and LexAop transgenes, and for advanced intersectional expression. We confirmed that a QF2wAD domain, when combined with GAL4DBD or ZpLexADBD, drives simultaneous expression from both UAS-red fluorescent protein and the LexAop-GFP reporters in both larvae and adults (Figure 3C). For advanced intersectional experiments, we regulated the expression of QS via the FLP-FRT system that, in turn, controlled the split transactivators. As expected, intersection of Chat-GAL4DBD, nsyb-QF2wAD, and GH146-FLP resulted in strong labeling of cholinergic octafactory projection neurons (Figure 3D, left). No labeling was observed when Chat-GAL4DBD was replaced by
the glutamatergic driver VGlut-GAL4DBD (not shown). Similarly, we observed expression throughout the brain and in optic lobes in the cholinergergic, but not glutamatergic (not shown), neurons that are targeted by 20C11-FLP (Chen et al. 2014) (Figure 3D, middle). Interestingly, intersection of VT009847-ZpLexADBD, nsyb-QFAD, and 20C11-FLP resulted in labeling of only one SEZ neuron (Figure 3D, right). These experiments demonstrate that split-QF can effectively achieve simultaneous and intersectional expression, narrowing down the expression patterns of split-GAL4, split-LexA, and FLP lines.

Finally, we applied the split-QF system to study physiology and behavior in Drosophila. To explore the usability of GAL4DBD + QFAD for electrophysiology, we performed whole-cell patch-clamp recordings from aCC and RP2 motoneurons of third-instar larvae. Neuronal depolarization was evoked through activation of UAS-ChR2 (Pulver et al. 2009) expressed in all motoneurons by VGlut-GAL4DBD, nsyb-QF2wAD or, in controls, VGlut-GAL4 (Figure 3E and Table S6). The number of action potentials produced from VGlut-GAL4DBD, nsyb-QF2wAD larvae (42 ± 6 per 500 msec) was not different from that observed in the GAL4 controls (51 ± 6, P = 0.62). QS completely eliminated ChR2-induced depolarization in tub-QS, VGlut-GAL4DBD, nsyb-QF2wAD larvae (Figure 3E), while feeding larvae of the same genotype with QA partially restored depolarization and action potential count (10 ± 5), but to a level significantly below the unrepressed levels of VGlut-GAL4DBD, nsyb-QF2wAD larvae (P = 0.0016). These readouts of cellular activity are paralleled by behavioral phenotypes. We counted how many times (in 2 min) larvae of these four genotypes escaped a blue-light area (Figure 3F and Table S6). As expected, larvae containing the QS transgene escaped most readily (11 ± 1.8 escapes), while feeding larvae with QA significantly reduced the number of escapes to 9.3 ± 1.3 (P = 0.038), due to the seizure-like neuronal activity elicited by ChR2 activation. VGlut-GAL4DBD, nsyb-QF2wAD were also able to escape (5.9 ± 0.6), but significantly less than the QS larvae (P < 0.0001). VGlut-GAL4 control larvae were unable to escape (0.2 ± 0.1).

We used the same assay to measure larval escape following activation of ChR2 driven pan-neuronally by split-QF (Figure 3G and Table S7). Abolished mobility was observed in larvae that expressed ChR2 (0 ± 0 escapes in nsyb-QFDBD, nsyb-QFAD and nsyb-QFDBD, nsyb-QF2wAD larvae), and in larvae that expressed QS and were fed with QA (0.3 ± 0.2 and 0.1 ± 0.1 escapes for QFAD and QF2wAD, respectively). By contrast, QS-expressing larvae not fed with QA readily escaped the blue-light area (7.4 ± 0.7 and 8.0 ± 0.8 escapes, respectively).

We also assayed adult flies with pan-neuronal expression of shibireTS (Figure 3H and Table S8). When placed at 33°C, nsyb-QFDBD, nsyb-QFAD flies became gradually paralyzed as expected. The same effect was observed in nsyb-QFDBD, nsyb-QF2wAD flies but took longer to develop, presumably due to the lower expression levels of shibireTS. When the expression of shibireTS was suppressed by tub-QS, no paralysis was observed.

Collectively, these experiments demonstrate that split-QF may be used with or without split-GAL4 to direct the expression of effectors in electrophysiological and behavioral assays.

Discussion

Here, we demonstrate the use of split-QF by itself, or in combinations with split-GAL4 and split-LexA, for advanced intersectional experiments, concurrent independent use of UAS and LexAop transactivators, electrophysiology, optogenetics, and thermogenetics in Drosophila. Ultimately, split-QF facilitates targeting of small populations of cells, and may be used for neuronal connectomics analysis or to explore behavioral phenotypes that are produced by artificial activation of single neurons. The absence of large libraries of split-QF lines is currently limiting, but these lines may be generated from split-GAL4 and split-LexA stocks by, for example, the HACK method (Lin and Potter 2016).

Quantification of transactivator activity

Three factors likely contribute to the lower strength of split transactivators when compared to the full-length ones (Figure 1, C and D and Figure 2, B and C). First, the nsyb-QFDBD transgene has been integrated into the attp40 site, which may be weaker than attp2, where the full transactivators are integrated. Second, the binding between QFDBD and QFAD (QF2wAD) is a process with a probability < 1, which is predicted to result in a lower number of reconstituted split transactivators compared to full-length ones. Consistent with this idea, the reporter expression by split transactivators appeared to be more variable than normally observed with full-length transactivators. Third, it is possible that the spatial configuration of the reconstituted QFDBD + QFAD (QF2wAD) is somewhat different and less efficient than the full-length QF2 (QF2w). Reduction of the transactivator strength is assessed by the expression level of reporters, which directly translates into the number of labeled cells as low levels of reporter expression could render some cells undetectable.

The luciferase and electrophysiology readouts of QA-fed GAL4DBD+QFAD/QF2wAD larvae were significantly below those of the nonsuppressed GAL4DBD+QFAD/QF2wAD larvae (Figure 2B and Figure 3E). On the other hand, GFP readouts of QA-fed LexA+QFAD/QF2wAD larvae and adults appeared to be equal to the unrepressed LexA+QFAD/QF2wAD (Figure 2D). These results, combined with the split-QF data (Figure 1C), indicate that the strength of QS binding to QFAD may be affected by the overall conformation of the reconstituted transactivator, subsequently resulting in different QA efficiency.

Use of split-QF beyond Drosophila

The first use of split-QF was reported in C. elegans (Wei et al. 2012), albeit in a different form, including a QF dimerization domain, and without detailed characterization of QS and QA.
Figure 2  Split-QF, split-GAL4, and split-LexA. (A) Top: expression of GFP in larval CNS, driven by elav-GAL4DBD and nsyb-QFAD (three left columns), or nsyb-QF2^AD (three right columns). Second and fifth columns show tub-QS-induced repression. Third and sixth columns show recovery of expression in larvae, grown on food with quinic acid (QA). Bar, 200 \( \mu \text{m} \). Bottom: same as top, but driven by VT019838-GAL4DBD in adult CNS. Adults were fed with QA for 5 days. Bar, 50 \( \mu \text{m} \). (B) Quantification of relative strength of chimeric split transactivator in larval CNS. Genotypes were elav-GAL4DBD, nsyb-QFAD, UAS-luc (red) or elav-GAL4DBD, nsyb-QF2^AD, UAS-luc (blue), without (left) or with (middle) tub-QS and QA treatment (right). elav-GAL4DBD, nsyb-GAL4AD, UAS-luc larvae (gray) had very low luciferase levels, while elav-GAL4DBD, nsyb-p65AD, UAS-luc larvae did not survive. Purple bars show data from nsyb-GAL4QF, UAS-luc larvae for comparison. (C) Same as (B), but in adult CNS. Males and females are quantified separately due to significantly different expression levels. Green data points show quantification for elav-GAL4DBD, UAS-luc; nsyb-QFAD, UAS-luc and nsyb-QF2^AD, UAS-luc controls. (D) top. Expression of GFP in larval CNS, driven by VT007395-LexADBD and nsyb-QFAD (three left columns), or nsyb-QF2^AD (three right columns). Second and fifth columns show tub-QS induced repression. Third and sixth columns show recovery of expression in the larvae, grown on food with QA. Bar, 200 \( \mu \text{m} \). (D) bottom. Same as top, but driven by VT009847-LexADBD in adult CNS. Adults were fed with QA for 5 days. Bar, 50 \( \mu \text{m} \).
Figure 3 Applications of split-QF. (A and B) Repression of expression by Killer-Zipper (Dolan et al. 2017) or tub-QS. Expression levels were quantified in adult flies using a luciferase assay. Genotypes of flies without repression were nsyb-QFDBD, nsyb-QFAD, QUAS-Luc (A, left) or elav-GAL4DBD, nsyb-QFAD, UAS-Luc (B, left). Killer-Zipper flies were nsyb-QFDBD, nsyb-QFAD, nsyb-LexAQF, lexAop-KZip+, QUAS-Luc (A, middle, green) or elav-GAL4DBD, nsyb-QFAD, nsyb-LexAQF, lexAop-KZip+, UAS-Luc (B, middle, green). QS flies were tub-QS, nsyb-QFDBD, nsyb-QFAD, QUAS-Luc (A, right) or tub-QS, elav-GAL4DBD, nsyb-QFAD, UAS-Luc (B, right). (C) Simultaneous expression of red fluorescent protein (RFP) and GFP in independent neuronal subpopulations in larval (left, bar, 200 µm) and adult (right, bar, 50 µm), by QF2wAD forming functional transactivators with GAL4DBD and LexADBD. (D) Intersectonal expression, enabled by QS-repressible GAL4DBD+QF/QF2wAD and LexADBD-QFAD transactivators. GFP is expressed in cells that: (1) are expressing FLP or are progeny of cells that were expressing FLP; (2) are expressing GAL4DBD or LexADBD; and (3) are expressing QFAD or QF2wAD. Third panel shows a zoomed-in image of the z-stack of the brain, shown in the second panel. Bar, 50 µm. (E) Whole-cell patch-clamp recordings from aCC/PR2 motoneurons in third-instar larvae of indicated genotypes, raised on food supplemented with all-trans retinal. Depolarization was elicited by blue light. Example traces are shown on the right. Bars (traces: 10 mV/100 msec, stimulus: 2 V/100 msec). (F) Escape assay of larvae with the same genotypes as in (E). Each larva was given 2 min to escape from a 113 mm2 area lit by blue light (λ470 nm). Once the larva had completely left the lit area, it was returned into the area. (G) Escape assay of nsyb-QFDBD, nsyb-QFAD, QUAS-ChR2 vae (red) and nsyb-QFDBD, nsyb-QF2wAD, QUAS-ChR2 larvae (blue), with or without tub-QS and quinic acid (QA). (H) Adult nsyb-QFDBD, nsyb-QFAD, QUAS-shiTS (red diamonds) and nsyb-QFDBD, nsyb-QF2wAD, QUAS-shiTS (dark-blue upward triangles) flies were paralyzed when placed in a 33°C incubator at t = 0 min. Flies that also had a tub-QS transgene (yellow squares and light-blue downward triangles) were not paralyzed. The data show the average number of flies (out of 10, ± SEM) at the bottom of the vial over time. Each graph is an average of n = 5 repeats, apart from “QF2wAD+QS,” with n = 4. Red and blue dots indicate the time point when the corresponding genotypes with and without QS became significantly different for the first time (Student’s t-test with Holm–Sidak correction for multiple comparisons). Stars indicate data points where nsyb-QFDBD, nsyb-QFAD, QUAS-shiTS and nsyb-QFDBD, nsyb-QF2wAD, QUAS-shiTS flies performed significantly differently (Student’s t-test with Holm–Sidak correction for multiple comparisons).
effects. Experiments presented here indicate that QF2\textsuperscript{AD}, QS, and QA are likely to be functional in *C. elegans*, and can extend the use of split-QF in this organism.

The Q-system works well across species. It has recently been introduced into zebrafish (Subedi et al. 2014) and malaria mosquitoes (Riabinina et al. 2016), dramatically expanding the very limited set of tools for transgene expression in mosquitoes. Split-QF may thus be a very useful addition to the genetic toolkit for these two organisms. Considering progressively wider use of genetic tools in other arthropods, such as moths (Long et al. 2015), beetles (Rylee et al. 2018), locusts (Wang et al. 2018), ants (Yan et al. 2017), and bees (Schulte et al. 2014), and in plants (Bruce et al. 2015), the split-QF and the Q-system may be the tools of choice in these organisms as well.

In summary, we present a split-QF system that is applicable for advanced anatomical, behavioral, and physiological manipulations in *Drosophila*. This system is fully compatible with and complementary to the existing split-GAL4 and split-LexA lines, and can greatly expand their use by making them QS-repressible and QA-inducible. In addition, combinations of split-QF with split-GAL4 and split-LexA systems can make extensive use of the available UAS and LexAop reporters.

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