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Note

Focusing Transgene Expression in Drosophila by Coupling Gal4 With a Novel Split-LexA Expression System

Chun-Yuan Ting,* Stephanie Gu,* Sudha Guttikonda,* Tzu-Yang Lin,* Benjamin H. White† and Chi-Hon Lee*,[1]

**Section on Neuronal Connectivity, Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892 and* †*Section on Neural Function, Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892*

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ABSTRACT

Here we report the development of a ternary version of the LexA::VP16/LexAop system in which the DNA-binding and *trans*-activating moieties are independently targeted using distinct promoters to achieve highly restricted, intersectional expression patterns. This Split LexA system can be concatenated with the Gal4/upstream activating sequence system to refine the expression patterns of existing Gal4 lines with minimal genetic manipulations.

BINARY expression systems, such as the Gal4/upstream activating sequence (UAS) system commonly used in Drosophila (Duffy 2002), typically exploit the unique DNA-binding properties of a heterologous transcription factor to drive effector transgene expression. In addition to Gal4/UAS, several other binary systems, including those based on the bacterial DNA-binding protein LexA (Lai and Lee 2006; Pfeiffer *et al.* 2010; Yagi *et al.* 2010) and the fungal transcription factor Q (Potter *et al.* 2010), have been developed recently for use in Drosophila. When used in combination, these different systems allow independent expression of several transgenes in a single animal.

A limitation of all binary systems is that the spatial and temporal patterns of transgene expression are dictated by a single promoter, which may not have the desired specificity. To generate more restricted expression patterns, combinatorial systems that make transgene expression dependent upon the activity of two promoters have been developed. Several techniques couple Gal4-mediated expression to an excision event mediated by an independently targeted recombinase, such as the yeast flipase or bacteriophage cre enzyme (Bohm *et al.* 2010). Because excision is irreversible, transgene expression in these systems reflects the cumulative pattern of activation of the recombinase over the developmental history of the organism. Such combinatorial systems are particularly useful for lineage tracing, but may also lack the desired specificity. In contrast, the "Split Gal4" system, which independently targets the Gal4 DNA-binding domain (Gal4DBD) and a cognate transcription activation domain (AD) using two different promoters, drives transgene expression in a temporally restricted fashion: only cells in which both promoters are active at the same time express the two heterodimerizing transcription factor domains to reconstitute transcriptional activity and drive transgene expression. The combined spatial and temporal specificity of the Split Gal4 system offers potential advantages in dissecting complex neural circuits such as those involved in wing expansion and phototaxis (Luan *et al.* 2006; Gao *et al.* 2008).

A disadvantage of the Split Gal4 system, however, is that it cannot be used with existing Gal4 lines. To refine the expression of an existing Gal4 driver line, one needs to generate a corresponding Gal4DBD or AD hemi-driver either by making new transgenic lines or by *P*-element swap (Sepp and Auld 1999). Making new lines is time-consuming and, depending on how a given line is made, the resulting hemi-driver may not always reproduce the original expression pattern. Here we report a new split expression system that can operate in parallel to the existing Gal4/UAS

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[1]*Corresponding author:* Section on Neuronal Connectivity, Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bldg. 18T, Room 106, Bethesda, MD 20892.
E-mail: leechih@mail.nih.gov

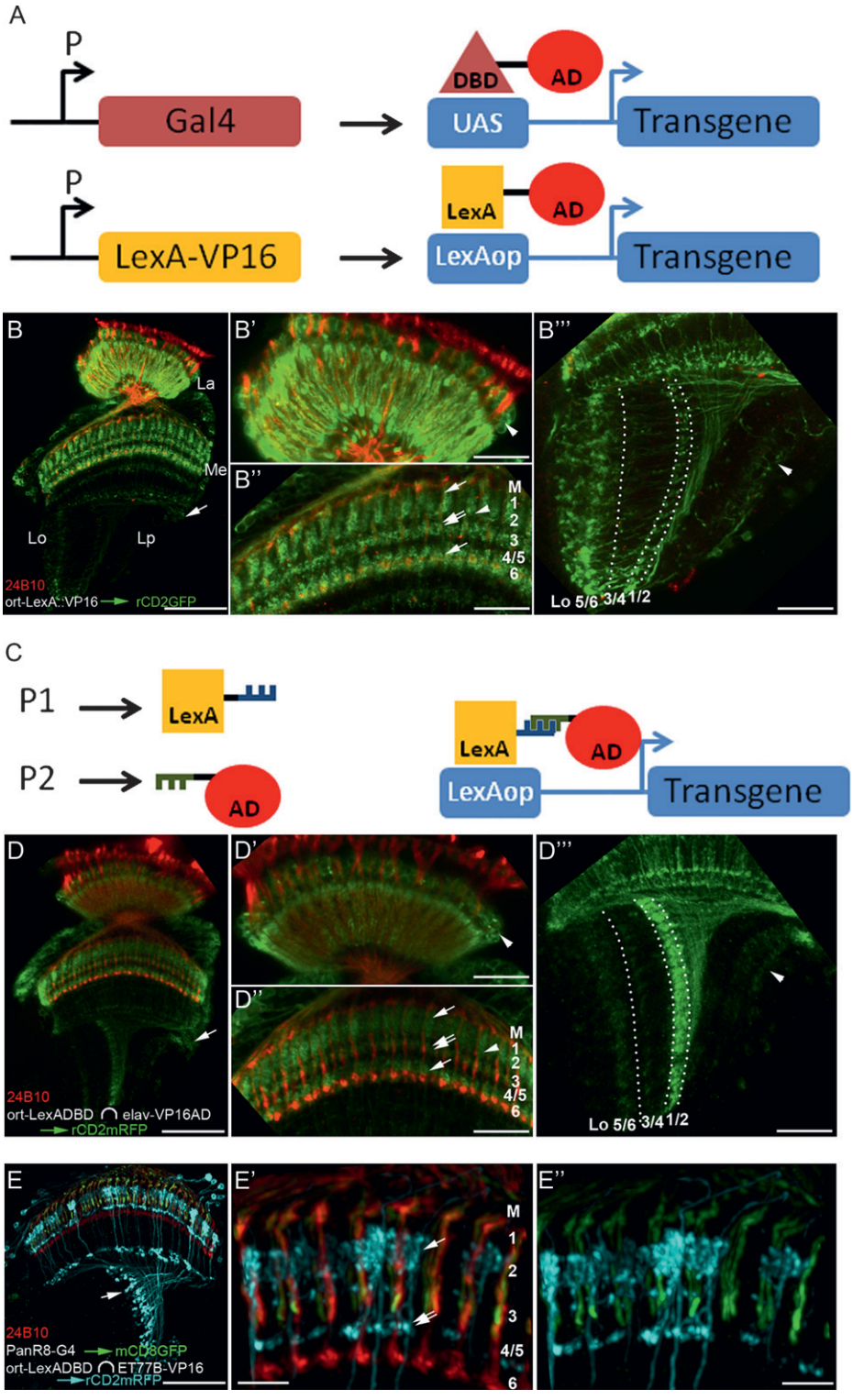

FIGURE 1.—The Split LexA system for restricting transgene expression to the intersection of the expression patterns of two promoters. (A) A schematic of the classic binary expression systems, Gal4/UAS and LexA::VP16/$LexA_{op}$. In the Gal4/UAS system, a promoter/enhancer (P) drives the expression of the yeast transcription factor Gal4, which binds to the UAS through its DNA-binding domain (DBD) activating transcription of a downstream transgene through its activation domain (AD). In the LexA::VP16/$LexA_{op}$ system, a promoter/enhancer (P) drives the expression of the *Escherichia coli* transcriptional repressor LexA fused to the VP16 *trans*-activation domain. LexA::VP16 then binds to the LexA operator sequence ($LexA_{op}$) through its DNA-binding domain (LexA) activating transcription of a downstream transgene through its VP16AD domain. (B) LexA::VP16 drives expression of transgenes downstream of $LexA_{op}$. The promoter of the Drosophila *ort* gene, which encodes a histamine receptor, drives expression in subsets of lamina (La) and medulla (Me) neurons in the visual system, some of which send projections into the lobula complex (Lo: lobula; Lp: lobula plate). The ort-LexA::VP16 driver induced the expression of a membrane-tethered GFP reporter (rCD2GFP, green) in these regions as well as in a subset of lobula neurons (C2/T2) (arrow). Photoreceptor axons stained with MAb24B10 (red) were used as landmarks. (B′–B′′′) High magnification views of B showing the lamina (B′), medulla (B′′), and lobula complex (B′′′; dotted lines outline the indicated layers of the lobula). The soma of three types of Ort-positive lamina neurons, L1–3, are labeled in the lamina cortex (arrowhead, B′), and their axonal termini are found in distinct medulla layers (L1: layers M1 and M5, arrows; L2: M2, arrowhead; L3: M3, double arrow, B′′). The axonal termini of Ort-positive medulla neurons were found in the Lo 1, 2, and 5 layers of the lobula (B′′′). (C) A schematic of the Split LexA system. In the Split LexA system, the DNA-binding domain (LexA) and a transcription AD are each fused to a different leucine zipper capable of heterodimerizing to its complement on the other domain (blue and green keys, and each can be expressed under the control of its own promoter (P1, P2). Only in cells that express both domains do the components heterodimerize and drive the expression of the transgene downstream of the $LexA_{op}$ promoter. (D–D′′′) The Split LexA system efficiently drives reporter expression *in vivo*. An ort-LexADBD hemi-driver was used in combination with the pan-neuronal hemi-driver elav-VP16AD to drive the expression of a membrane-tethered RFP reporter (rCD2mRFP, pseudocolored in green). Antibody staining (red) is as in B. While the relative level of expression in different cell types varied, the Split LexA drivers ort-LexADBD ∩ elav-VP16AD recapitulated the expression of the conventional ort-LexA::VP16 driver shown in B and included C2/T2 neurons (arrow, D). (D′–D′′′) High-magnification views of D showing the lamina (D′), medulla (D′′), and lobula complex (D′′′). As in B, lamina neurons L1–L3 (D′ and D′′, labeled as in B′ and B′′) and a subset of medulla neurons (as indicated by

system and is based on the bacterial LexA protein, which has DNA-binding but not *trans*-activation, activity. An advantage of this split LexA system is that it can also function in series with the Gal4/UAS system to refine the expression patterns of existing Gal4 lines. A UAS-LexADBD construct can be used in conjunction with a Gal4 driver and diversely targeted cognate VP16AD domains to dissect the expression pattern of the Gal4 line. We call this implementation of the Split LexA system "concatenation."

To develop the Split LexA system, we first made heterodimerizing LexADBD and VP16AD constructs by fusing each of these domains to one of the two complementary leucine zippers (zip) from the Split Gal4 system (supporting information, File S1, Figure S1). We then tested the efficacy of the resulting "zipLexADBD" and "VP16ADzip" constructs in Drosophila S2 cells. When co-expressed, the two complementary domains drove strong expression of a $LexA_{op}$-GFP reporter (Figure S2). Expression required the presence of both domains (Figure S2, A *vs.* G and H) and, as with the original Split Gal4 system (Figure S2F), the Gal4 activation domain could replace the VP16AD (Figure S2B). In addition, we found that reporter expression could be effectively driven by concatenating Gal4 with a UAS-LexADBD construct (Figure S2, C and D).

To test the efficacy of the Split LexA system *in vivo*, we generated transgenic flies that expressed zipLexA in second-order visual neurons using the *ort* promoter (ort-LexADBD; Table S1). The *ort* promoter has been well characterized and is known to be active in a subset of lamina and medulla neurons, most of which receive direct synaptic inputs from photoreceptors, as shown with a conventional ort-LexA::VP16 binary driver in Figure 1, B–B′′′ (Gao *et al.* 2008). Combining the ort-LexADBD hemi-driver with a pan-neuronally expressed activation domain (*i.e.*, elav-VP16AD) produced an equivalent pattern of expression of a $LexA_{op}$-rCD2mRFP reporter (Figure 1, D–D′′′). While the overall expression level of ort-LexADBD ∩ elav-VP16AD is somewhat lower than that of ort-LexA::VP16, the red fluorescence protein (RFP) reporter labeling has a high degree of specificity across the entire lamina and medulla. For example, three well-characterized Ort-positive lamina neurons, L1–L3, are RFP-labeled across the entire lamina field, as well as in the medulla as judged by the presence of their axon terminals in medulla layers (L1: M1 and M5; L2: M2; L3: M3). Interestingly, the uniformity of labeling by the ort-LexADBD ∩ elav-VP16AD driver differs from the heterogeneous labeling seen with ort-Gal4. It is not clear whether this is due to the nature of these factors, the strength of *trans*-activation domains, or both. The presence of Ort-positive transmedulla neurons, such as Tm2, Tm9, Tm5, and Tm20, was also evident on the basis of the homogenous labeling of axonal terminals in the lobula (Figure 1D′′′). However, the identity of the labeled cells could not be uniquely ascertained on the basis of the pattern of labeling in the lobula because these transmedulla neurons project their axons to overlapping lobula layers (Tm9: Lo1; Tm2: Lo2; Tm5a/b/c and Tm20: Lo5).

To more rigorously test the specificity of the ort-LexADBD hemi-driver, we compared its performance to the previously characterized ort-Gal4DBD, which, when combined with the ET77B-VP16AD enhancer trap line, clearly labels only one type of transmedulla neuron, Tm2 (Figure 1, E–E′′). Similar to ort-Gal4DBD, the ort-LexADBD hemi-driver labels, in conjunction with ET77B-VP16AD, labeled the unique Tm2 dendritic arbors in the medulla layers M2–4 and the Tm2 axon terminals in the lobula layer Lo2 (Figure 1, E–E′′, and data not shown). Interestingly, labeling of Tm2 neurons using ort-LexADBD also duplicated the labeling seen with the ort-Gal4DBD in its variability. In contrast to the homogeneity of labeling seen in the L1–L3 neurons, the Tm2 neurons were inconsistently labeled across the medulla. Whether this inconsistency reflects true stochastic activity of the ort promoter or is an artifact of the expression systems is not clear.

To test for potential cross-reactivity between the Gal4 and Split LexA systems, we co-expressed, in flies bearing the ort-LexADBD, ET77B-VP16AD, and $LexA_{op}$-rCD2mRFP transgenes, a PanR8-Gal4 driver and a UAS-mCD8GFP reporter, which should label the R8 photoreceptors. The complete absence of double labeling demonstrates that the Split LexA and Gal4 systems function independently without any detectable cross-reactivity (Figure 1, E–E′′). In summary, on the basis of our results of corresponding ort promoter constructs, the Split LexA and Split Gal4 systems seemed to have similar specificity and efficacy. Because the Split LexA system also functions orthogonally to the Gal4/UAS system, it should also be possible to use the Split LexA system to refine the expression patterns of known Gal4 drivers.

labeling of processes in various lobula layers in D′′′) were labeled. A few lobula plate neurons were also labeled (arrowhead, D′′′) at a low level, as seen in B′′′. (E–E′′) The Split LexA system can be used to refine the *ort* expression pattern. By combining the ort-LexADBD hemi-driver with a VP16AD enhancer trap line, ET77B, a single population of Ort-positive neuron, namely the Tm2 transmedulla neurons, can be labeled by the rCD2mRFP reporter (pseudocolored in cyan). The Tm2 neurons were identified by their axonal termini at the lobula layer Lo2 (arrow, E) and by their unique patterns of dendritic processes in the medulla layers M2 and M4 (arrow and double arrows, respectively, E′). A panR8-Gal4 driver and a UAS-mCD8GFP reporter were also included to label all R8 photoreceptors (green). The Gal4 and Split LexA systems maintain their expression patterns without any detectable cross-reactivity. (For clarity, the red channel was removed in E′′). Scale bar: 50 μm in B, D, and E; 20 μm in B′–B′′′ and in D′–D′′′; and 10 μm in E′–E′′.

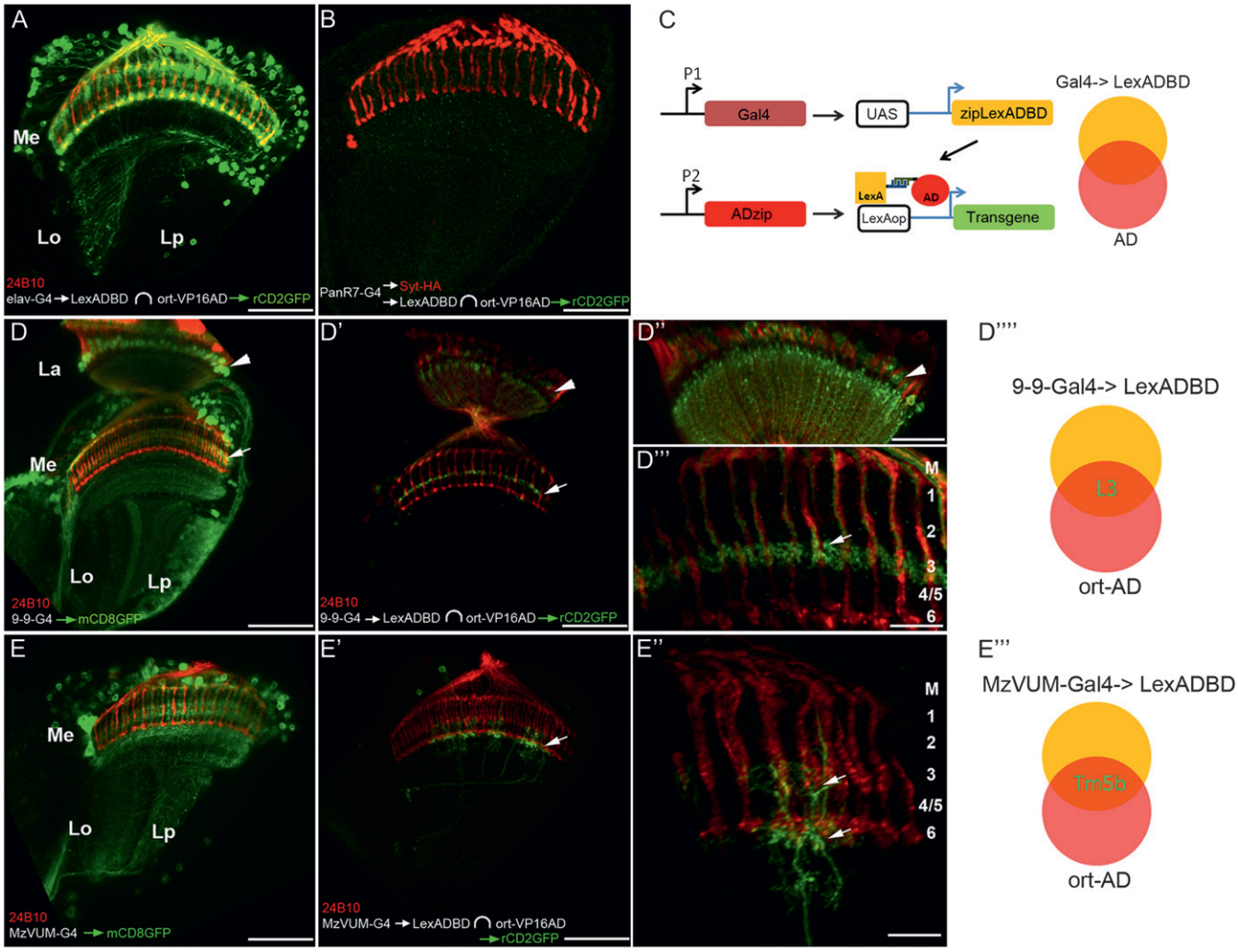

FIGURE 2.—Restriction of expression can be achieved by concatenating the Gal4 and Split LexA systems. (A) The pan-neuronal driver elav-Gal4 was used to express zipLexADBD, which in combination with the hemi-driver ort-VP16AD drives expression of a membrane-tethered GFP reporter (rCD2GFP, green). This concatenated combination recapitulates the ort expression pattern as seen in Figure 1, B and D. Photoreceptors axons, visualized by the MAB24B10 antibody (red), were used as landmarks. (B) To test the stringency of the concatenated expression system, the PanR7-Gal4 driver and the ort-VP16AD hemi-driver, which have no overlapping expression, were combined with UAS-LexADBD as in A. No rCD2GFP (green) reporter expression was detected in the concatenated system. As an internal control, a UAS-syt-HA reporter transgene was included to reveal the expression pattern of the PanR7-Gal4 driver in R7 photoreceptors (red). (C) A schematic of the Split LexA concatenated expression system. In this system, a UAS-LexADBD transgene is used to couple the Gal4/UAS and the Split LexA system. The promoter P1, via Gal4, drives the expression of zipLexADBD, which, in combination with the ADzip driven by the second promoter P2, leads to the expression of the $LexA_{op}$ transgene at the intersection of the P1 and P2 expression patterns. (D) The expression pattern of the 9-9-Gal4 enhancer trap line revealed by the expression of a GFP reporter (mCD8GFP, green). 9-9-Gal4 expresses in several subsets of optic lobe neuron, including the Ort-positive lamina neurons, L3 (cell body, arrowhead; axonal terminal, arrow, D, D′, and D′′′). (D′–D′′′) The combination of 9-9-Gal4 → LexADBD ∩ ort-VP16AD) drives reporter expression in the lamina L3 neurons. (D′′ and D′′′) High magnification views of the lamina and medulla neuropils of D′, respectively. (D′′′′) A Venn diagram representation of 9-9-G4 → LexADBD ∩ ort-VP16AD. (E) The expression pattern of the MzVUM-Gal4 enhancer trap line as revealed by mCD8GFP expression (green). (E′ and E′′) The transmedulla Tm5b neurons (arrows) were labeled by MzVUM-G4 → LexADBD ∩ ort-VP16AD. (E′′) A high-magnification view of the medulla neuropil of E′. (E′′′) The expression of the concatenated driver MzVUM-G4 → LexADBD ∩ ort-VP16AD shown as a Venn diagram. La: lamina; Me: medulla; Lo: lobula; Lp: lobula plate. Scale bar: 50 μm in A, B, D, D′, E, and E′; 20 μm in D′′ and D′′′; and 10 μm in E′′.

To test this possibility, we used a UAS-LexADBD transgene to couple the Split LexA and Gal4 systems. In this configuration, Gal4 drives the expression of LexADBD, which, in combination with a VP16AD hemi-driver, will drive $LexA_{op}$-transgene expression. Transgene expression, however, is now limited to cells that express both Gal4 and VP16AD (Figure 2C). As a proof-of-concept, we first showed that the concatenated system could recapitulate the Ort expression pattern. We used elav-Gal4 to express LexADBD in all neurons and,

together with the ort-VP16AD hemi-driver, to drive expression of $LexA_{op}$-rCD2GFP in Ort-expressing neurons (Figure 2A). As a negative control, we used PanR7-Gal4 to express LexADBD in all R7 photoreceptors. The latter components, in combination with the ort-VP16AD hemi-driver, produced no detectable reporter expression (Figure 2B), again confirming the orthogonality of the Gal4 and Split LexA systems.

We next tested whether the concatenated system can refine the expression pattern of existing Gal4 lines. We tested two enhancer trap Gal4 lines, 9-9-Gal4 and MzVUM-Gal4, which drive reporter expression in two Ort-positive neurons, L3 and Tm5b, respectively (Erclik *et al.* 2008 and our unpublished data). In addition, both lines drive expression in numerous Ort-negative lamina and medulla neurons (Figure 2, D and E, and unpublished data). In the concatenated configuration (9-9-G4→LexADBD ∩ Ort-VP16AD), the combination of 9-9-Gal4 and ort-VP16AD selectively drove reporter expression in the lamina L3 neurons (Figure 2, D′–D′′′), while the combination of the MzVUM-Gal4 and ort-VP16AD (MzVUM-G4→LexADBD ∩ Ort-VP16AD) drove reporter expression in a single type of medulla neurons, Tm5b (Figure 2, E′–E′′). These results demonstrate that the Split LexA-based concatenated expression system can substantially simplify the complex expression patterns of existing Gal4 lines, in some cases refining a heterogeneous pattern to one consisting of a single cell type.

In summary, we have developed an intersectional Split LexA expression system, in which $LexA_{op}$ transgene expression is made contingent upon the activity of two promoters. This system uses the same leucine zippers as the Split Gal4 system and can thus take advantage of existing libraries of VP16ADzip enhancer trap lines. We have also begun to generate a LexADBD enhancer trap line, which can likewise be used in conjunction with VP16AD lines to generate restricted expression patterns (Figure S3). Dual applications that take advantage of the orthogonality of the Gal4 and LexA systems should also be possible. For example, by using distinct Gal4DBD and LexADBD lines in conjunction with the same VP16AD line, one can, in principle, differentially manipulate distinct subsets of cells within the same pattern.

In addition to operating in parallel with the Gal4/UAS system, the Split LexA system can also operate in series with Gal4 drivers as a concatenated expression system. As we demonstrate using the Drosophila visual system as an example, the concatenated system is capable of refining complex Gal4 expression patterns in the optic lobe to homogeneous patterns containing a single cell type. This approach circumvents the need to convert existing Gal4 enhancer lines to the Split Gal4 system (Gao *et al.* 2008) and complements other methods for restricting the expression patterns of Gal4 drivers (Bohm *et al.* 2010). Over the past decade, thousands of Gal4 lines have been generated and many of their expression patterns have been characterized. By allowing the expression patterns of these lines to be rationally refined in a straightforward way, the concatenated expression system greatly facilitates the use of these resources.

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FILE S1

Supporting Material and Methods

Fly Stock

Fly stocks were maintained at 22°C on standard medium. The following stocks were used in this study: (1) *ort-LexADBD*, (2) *ort-LexA::VP16* (GAO *et al.* 2008), (3) *ort-VP16AD* (GAO *et al.* 2008), (4) *elav-VP16AD* (LUAN *et al.* 2006), (5) *OK371-dVP16AD* (GAO *et al.* 2008), (6) *elav-G4DBD* (LUAN *et al.* 2006), (7) *elav*-VP16AD (LUAN *et al.* 2006), (8) *ET-LexADBD* enhancer trap, (9) *ET-VP16AD* enhancer trap (LUAN *et al.* 2006), (10) *elav (C155)-Gal4* (Bloomington stock center), (11) *MzVUM-Gal4* (ERCLIK *et al.* 2008), (12) *9-9-Gal4* (gift from Thomas Clandinin), (13) *PanR8-Gal4* (gift from Claude Desplan), (14) *PanR7-Gal4* (TING *et al.* 2005), (15) *UAS-LexADBD*, (16) *UAS-Syt-HA* (gift from Liquin Luo), (17) *UAS-mCD8GFP* (Bloomington stock center), (18) $LexA_{op}$*-rCD2GFP* (LAI and LEE 2006), (19) $LexA_{op}$*-rCD2mRFP* (LAI and LEE 2006).

Molecular biology

The pActPL-LexADBD, pUAS-LexADBD, pOrt-LexADBD and p{y+, LexADBD} enhancer trap vectors (supplementary figure S1) were constructed using PCR-based or regular cloning techniques and confirmed by sequencing. Cloning procedures are described as below:

Construction of pActPL-LexADBD:

Full-length LexA coding region was PCR amplified from pLOT-LexA::VP16 (LAI and LEE 2006) and replacing the Gal4DBD of pActPL-Ga4DBD with 5'-SpeI to 3'-AscI restriction sites to create pActPL-LexADBD. The protein sequence of zipLexADBD is shown in Figure S1E.

Forward: 5'- CGACTAGTAAAGCGTTAACGGCCAG-3'

Reverse: 5'- TGGCGCGCCTTACAGCCAGTCGCCGTT-3'

Construction of pUAST-LexADBD:

ZipLexADBD fragment was isolated from SpeI and AscI digested pActPL-LexA construct and cloned into X11 (Luan et al., 2006) to create X11-LexADBD. Subsequently, X11-LexA was digested with EcoRI and StuI and the purified zip-LexA fragment was subcloned into pUAST to create pUAST-LexADBD.

Construction of p{y+, LexADBD}:

ZipLexADBD-SV40 termination fragment was PCR amplified from X11-LexADBD vector and subsequently cloned into PCR-blunt vector to create an intermediate vector PCR-blunt-LexADBD. A NotI fragment from PCR-blunt-LexADBD which contained zipLexADBD-SV40 termination fragment was cloned into p{y+, Gal80} (gift from Christopher Potter) to create p{y+, LexADBD}.

Forward: 5'- TGGATCCCAAAATGCTGGAGATCCGCGCCGC-3'

Reverse: 5'- TGCGGCCGCGATCCAGACATGATAAGATACATTG-3'

Construction of pC-attB-ORT-LexADBD:

PCR-blunt-LexADBD vector (see above) was digested with BamHI and NotI and replacing the Gal4-hs70 termination signal of pC-attB-ORT-Gal4 to create pC-attB-ORT-LexA vector. pC-attB-Gal4 vector is derived from pChs-Gal4 vector (APITZ 2002). Briefly, the attB fragment was PCR amplified from pUASTP2 (BATEMAN *et al.* 2006) and cloned into 5'-EcoRI to 3'-KpnI site and hs43 mini promoter was removed to create pC-attB-Gal4 vector.

Other vectors:

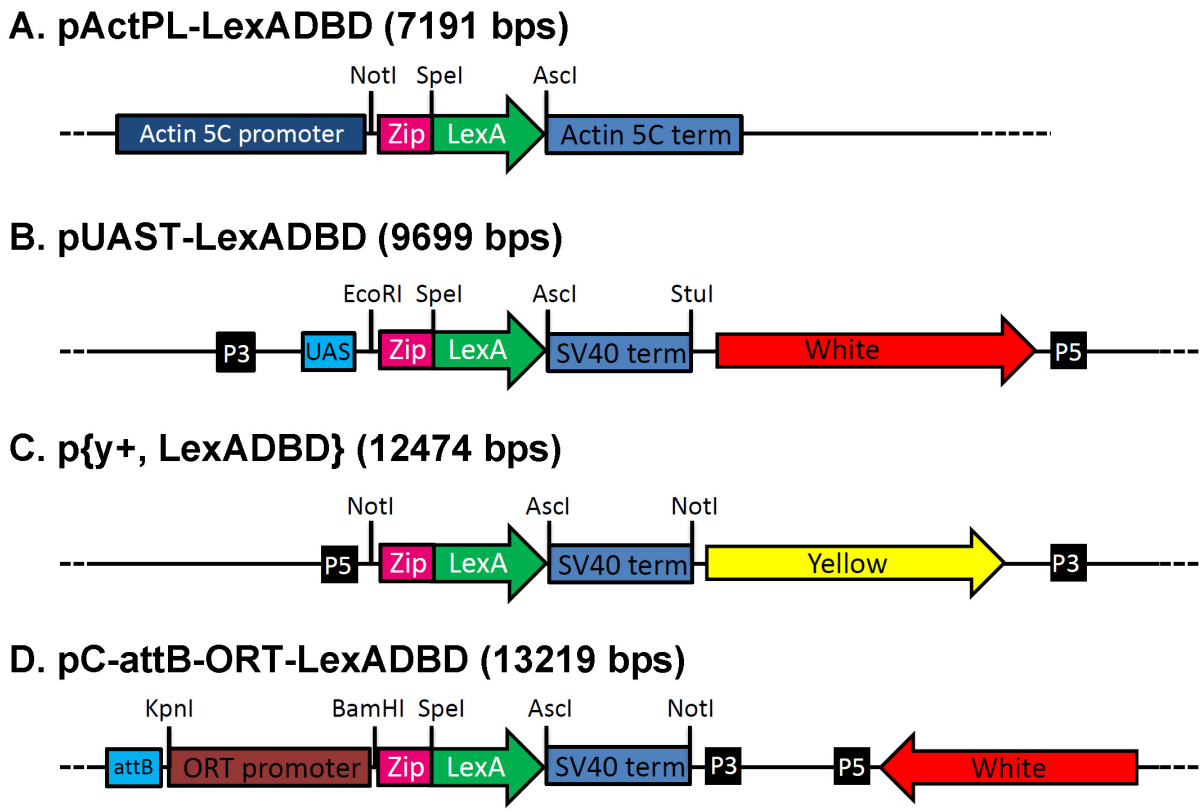
pActPL-Gal4, pActPL-GalDBD, pActPL-Gal4AD and pActPL-VP16AD vectors were described previously (LUAN *et al.* 2006).

Histology

Immunohistochemistry, confocal imaging, image deconvolution, and 3D image rendering were performed as described previously (TING *et al.* 2005). The following concentrations of primary antibodies were used: 24B10 (DSHB), 1:100 dilution; mouse anti-GFPMab (IgG2a, Invitrogen), 1:200 dilution; rat anti-CD2 (Serotec), 1:200 dilution; rat anti-HA (Roche), 1:200 dilution; rabbit anti-GFP, 1: 500 dilution (Torrey Pines Biolabs). The secondary antibodies including goat anti-rabbit, rat or mouse IgG coupled to Alexa 488, Alexa 568, or Alexa 647 (Invitrogen) were used in a 1:400 dilution.

Transfection

S2-adhesion cells were a generous gift from James Clemens. Cell culture and transfection with Lipofectamine 2000 were performed according to the Invitrogen manual.



E. zip::LexADBD

MLEIRAAFLRQRNTALRTEVAELEQEVQRLENEVSQYETRYGPLGGKASGGGGGGGGGGTSKALTARQQEVFDLIR
DHISQTGMPPTRAEIAQRLGFRSPNAAEEHLKALARKGVIEIVSGASRGIRLLQEEEEGLPLVGRVAAGEPLLAQQHIE
GHYQVDPSLFKPNADFLLRVSGMSMKDIGIMDGDLLAVHKTQDVRNGQVVVARIDDEVTVKRLKKQGNKVELLPEN
SEFKPIVVDLRQQSFTIEGLAVGVIRNGDWL*

FIGURE S1.—Schematic representations of (A) pActPL-LexADBD, a cell culture transfection vector; (B) pUAST-LexADBD, (C) p{y+, LexADBD} enhancer trap, and (D) pC-attB-ORT-LexADBD constructs. The *ort* promoter region can be replaced by other promoter through Kpn I and Bam HI cloning sites. (E) Amino acids sequence of zip::LexADBD. Color code: magenta for zip; green for full-length LexA protein. The poly-G linker is underlined.

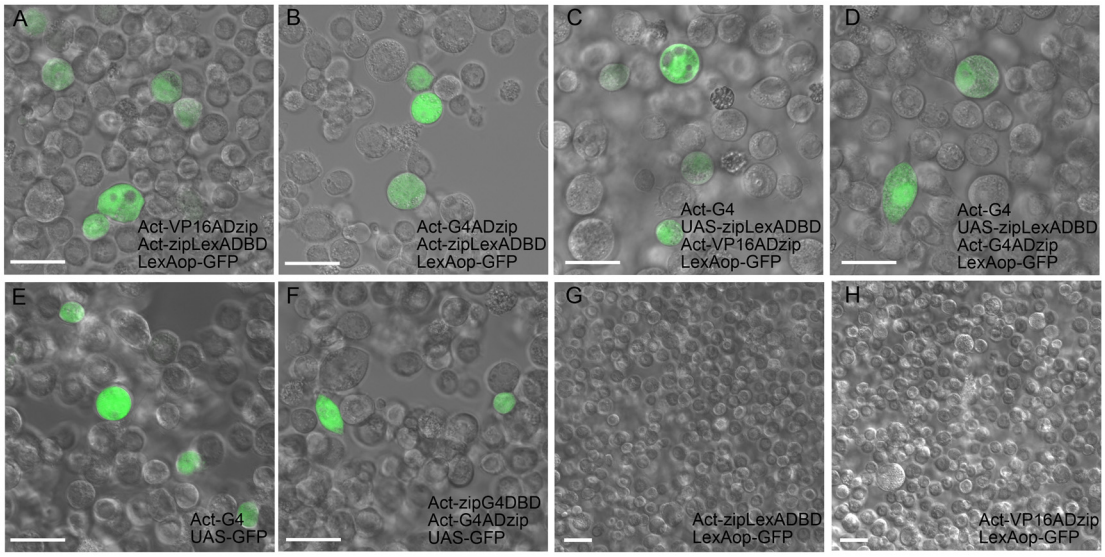


FIGURE S2.—Validation of the Split LexA system in cultured *Drosophila* S2 cells. (A-B) S2 cells were co-transfected with plasmids containing a zipLexADBD gene and either the VP16ADzip (A) or the Gal4ADzip (B) genes, all under the control of the Actin5C promoter. Transcriptional activity was evaluated by monitoring expression of a co-transfected $LexA_{op}$-GFP reporter construct (green fluorescence). (C-D) Co-transfection of Actin5C-Gal4, UAS-zipLexADBD, and Actin5C-VP16ADzip (C) or Actin5C-Gal4ADzip (D) demonstrating that Gal4 activity can be converted into LexA-mediated transcriptional activity. Gal4-mediated expression of zipLexADBD allows this component to complex with either the VP16ADzip (C) or Gal4ADzip (D) component to activate expression of a $LexA_{op}$-GFP reporter. (E) Gal4 protein drives UAS-GFP expression in S2 cells (positive control). (F) The Split Gal4 constructs, zipGal4DBD and Gal4ADzip, drive UAS-GFP expression in S2 cells (positive control). (G-H) S2 cells were transfected with a $LexA_{op}$-GFP reporter construct and either Actin5C-zipLexA (G), or Actin5C-VP16ADzip (H). The absence of GFP expression in both cases indicates that a single hemi-driver alone is insufficient to drive $LexA_{op}$-transgene expression *in vitro* (negative control). Scale bar: 20 μm in (A-H).

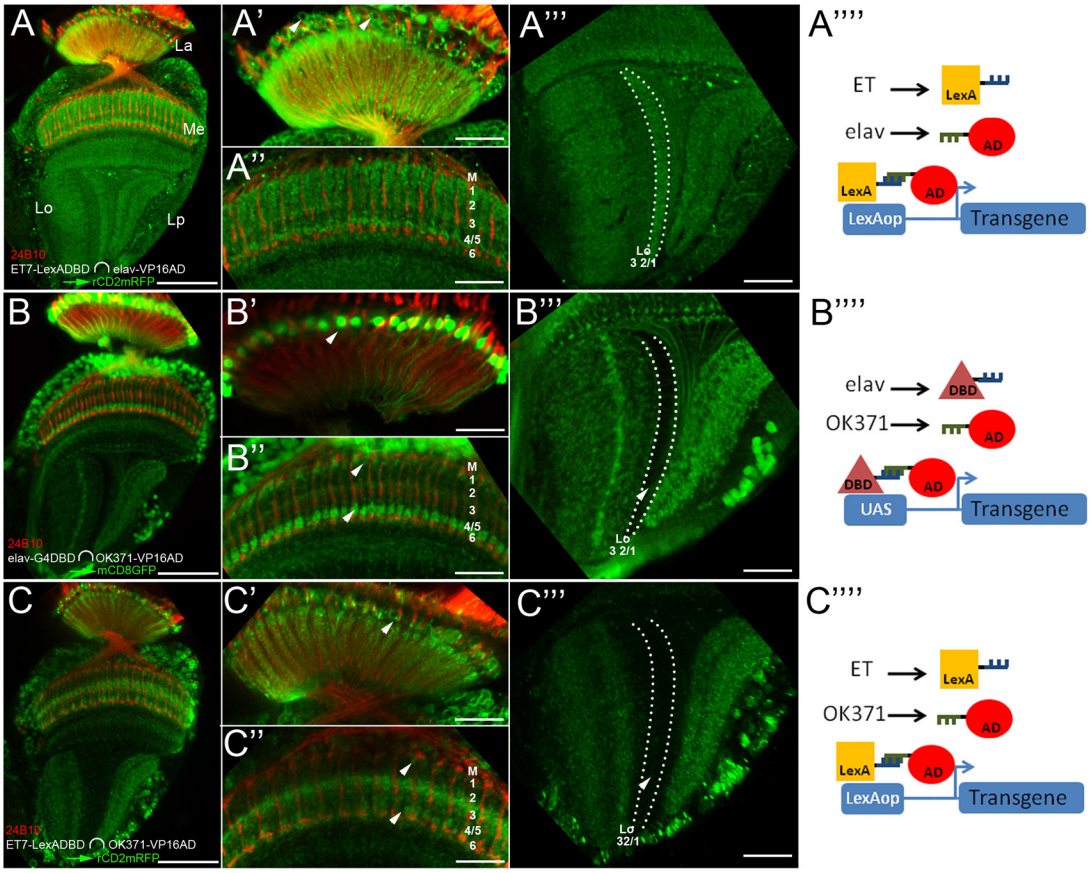


FIGURE S3.—Enhancer trap LexADBD hemidrivers can also be used in the Split LexA system. (A-A''') The expression pattern of the ET7-LexADBD enhancer trap line in the optic lobe was revealed by combining it with the pan-neuronal hemidriver elav-VP16AD (Elav-VP16AD∩ET7-LexA) to drive the expression of the rCD2mRFP reporter (pseudo-colored in green). Photoreceptor axons visualized with MAb24B10 (red) were used as landmarks. (A'-A''') High-magnification views of (A) showing the lamina (A'), medulla (A''), and lobula complex (A'''). ET7-LexA labeled subsets of lamina (including L1, arrowhead, A') and medulla neurons (including those projecting axons to the lobula layers Lo1 and 2 [A''']). (B-B''') The expression pattern of the OK371-VP16AD enhancer trap line in the optic lobes was revealed by combining it with the pan-neuronal hemidriver Elav-Gal4DBD to drive the expression of the mCD8GFP reporter (green). This Split Gal4 driver labeled presumptive glutamatergic neurons, including the lamina L1 neurons (cell body: arrow head, B'; axonal termini in the medulla M1 and M5 layers, arrowhead, B''), but not the lobula Lo1 and Lo2 layers (B''', arrow head). (C-C''') The intersectional pattern produced by the double enhancer trap combination OK371-VP16AD∩ET7-LexADBD (rCD2mRFP, pseudo-color in green). Note that RFP is expressed in the lamina L1 neurons (C', C''), but not in the lobula Lo1 and Lo2 layers (C'''). (B'-B''') and (C'-C''') are high magnification views of (B-C) as (A'-A''') for (A). (A'''',B'''',C'''') Schematic representations of the Split drivers and reporters in (A-C). Scale bar: 50 μm in (A-C); 20 μm in (A'-A''', B-B''', C-C''').

TABLE S1

Summary of Experimental Genotypes

Figures	Genotype
1B-B'''	*w; ort-LexA::VP16 / + ; $LexA_{op}$-rCD2GFP/ +*
1D-D'''	*yw; elav-VP16AD/ ort-LexADBD ; $LexA_{op}$-rCD2mRFP/ +*
1E-E''	*w; ET77BVP16AD, ort-LexADBD/ PanR8-G4; UAS-mCD8GFP/ $LexA_{op}$-rCD2mRFP*
2A	*elav(c155)-Gal4/ w; ort-VP16AD / + ; UAS-LexADBD/ $LexA_{op}$-rCD2GFP*
2B	*PanR7-Gal4, UAS-HAsyt/ w; ort-VP16AD/+ ; UAS-LexADBD, $LexA_{op}$-rCD2GFP/+*
2D	*yw; UAS-mCD8GFP / + ; 9-9Gal4 / +*
2D'-D'''	*w; UAS-LexADBD / ort-VP16AD ; 9-9Gal4 / $LexA_{op}$-rCD2GFP*
2E	*MzVUM-Gal4/ w; UAS-mCD8GFP / +*
2E'-E''	*MzVUM-Gal4/ w; ort-VP16AD / + ; $LexA_{op}$-rCD2GFP, UAS-LexADBD*
S3A-A'''	*w; elav-VP16AD/+ ; ET7-LexADBD / $LexA_{op}$-rCD2mRFP*
S3B-B'''	*yw; OK371-VP16AD / UAS-mCD8GFP ; elav-DBD / +*
S3C-C'''	*yw; OK371-VP16AD / + ; ET7-LexADBD / $LexA_{op}$-rCD2mRFP*