Published in final edited form as:

Genesis. 2010 January ; 48(1): 3–7. doi:10.1002/dvg.20571.

Characterization of a Dorsal-Eye Gal4 Line in *Drosophila*

Clayton M. Morrison¹ and Georg Halder^{1,2,3,*}

¹Program in Developmental Biology, Baylor College of Medicine, Houston, Texas

²Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, Texas

³Graduate School of Biomedical Sciences, Program in Genes and Development, University of Texas M. D. Anderson Cancer Center, Houston, Texas

Summary

In Drosophila, the Gal4-UAS system is used to drive ectopic gene expression in a tissue-specific manner. In this system, transgenic flies expressing tissue specific Gal4 are crossed to a line in which the gene to be expressed is under the control of a Gal4-responsive UAS sequence. The resulting progeny express the gene of interest in the pattern of the particular Gal4 line. Since a given UAS-transgene can be driven by any Gal4 line, this system is predominantly limited by available Gal4 lines. Here we report the characterization of a novel line, *DE-Gal4*, which in the eye is expressed in the dorsal compartment for the majority of development. Furthermore, we use functional tests to show that the *DE-Gal4* line is a useful tool with which to manipulate gene expression in half of the developing eye.

Keywords

Drosophila; UAS-Gal4; eye; overexpression; Hippo

INTRODUCTION

Manipulation of tissue-specific gene expression allows one to perform sophisticated genetic experiments. At its most basic level, tissue-specific overexpression can be used simply to overexpress a gene in a specific spatiotemporal pattern, allowing assessment of gain-of-function phenotypes. When coupled with either RNA interference (RNAi) or a recombination system such as Flp-FRT (Xu and Rubin, 1993) or Cre-loxP (Kos, 2004), tissue-specific loss-of-function can also be achieved.

In Drosophila, tissue-specific overexpression is generally performed with the Gal4-UAS system (Brand and Perrimon, 1993). In this system, two separate transgenes are required. The first transgene expresses the yeast transcription factor Gal4 under the control of a tissue-specific promoter. The second transgene bears the gene to be expressed under the control of a Gal4-responsive Upstream Activating Sequence, or UAS. The UAS-transgene is inactive in the absence of Gal4, thus the gene of interest is only expressed when both transgenes are present. A critical feature of this system is that Gal4 activates the UAS-transgene *in trans*, allowing a single UAS-transgene to be driven by any Gal4 line. Thus, the main factor that

limits the power and precision of the system is the availability of Gal4 lines expressing in useful patterns.

For the study of the developing wing disc, several Gal4 lines are available. Of particular utility are those which express Gal4 in a compartment-specific manner such as *apterous-Gal4* (dorsal compartment) (Calleja *et al.*, 1996), *cubitus interruptus-Gal4* (anterior compartment) (Croker *et al.*, 2006), *hedgehog-Gal4* and *engrailed-Gal4* (posterior compartment) (Harrison *et al.*, 1995; Tanimoto *et al.*, 2000). For example, we and others used *engrailed-Gal4* to drive UAS-transgenes in the posterior compartment and tested whether this overexpression affected the growth of clones mutant for other genes (Nolo *et al.*, 2006; Prober and Edgar, 2000). This strategy allowed direct comparison of the growth phenotypes of mutant clones (anterior compartment) versus mutant clones overexpressing the gene of interest (posterior compartment). Thus, compartment-specific Gal4 lines are very useful as tools to generate clearly delineated experimental and control tissues within a single imaginal disc.

To our knowledge, there are no available Gal4 lines allowing similar approaches in the developing eye. Indeed, two of the most frequently used eye-expressing Gal4 lines, *eyeless-Gal4* (Hazelett *et al.*, 1998) and *GMRGal4* (Hay *et al.*, 1994), express in early and late eye development, respectively. Thus, although they each drive expression in a subset of eye cells, direct comparison of transgene-expressing and -nonexpressing cells in single discs with these drivers is problematic because the experimental and control populations of cells are in different developmental stages. To generate a compartment-specific Gal4 in the eye, we focused on members of the Iroquois complex, *mirror*, *araucan*, and *caupolican*, which are expressed specifically in the dorsal half of third instar eye discs (Cavodeassi *et al.*, 1999; Kehl *et al.*, 1998; McNeill *et al.*, 1997). These genes encode homeobox transcription factors that specify regions of the developing fly including the dorsal half of the eye and the lateral region of the notum (Cavodeassi *et al.*, 1999, 2001; Gomez-Skarmeta *et al.*, 1996; Kehl *et al.*, 1998; McNeill *et al.*, 1997). Enhancer trap insertions into the Iroquois gene region frequently show strong dorsal-eye reporter expression, and little or no ventral expression (Choi *et al.*, 1996; McNeill *et al.*, 1997; Netter *et al.*, 1998). For example, a *P-lacW* enhancer trap line *mirr^{B1-12}* shows dorsal-eye-specific expression of *LacZ* in third instar discs (Choi *et al.*, 1996; Kehl *et al.*, 1998). In fact, even the *white⁺* transgene reporter, which normally produces red pigment uniformly in the eye, displays dorsal-specific expression in *mirr^{B1-12}* (Kehl *et al.*, 1998). Thus, we chose *mirr^{B1-12}*, as a starting point to make a Dorsal-Eye Gal4 line.

RESULTS AND DISCUSSION

We generated a Dorsal-Eye Gal4 line (*DE-Gal4*) via a P-element exchange procedure (Sepp and Auld, 1999), in which we replaced the *LacZ*-expressing *mirr^{B1-12}* P-element with a *Gal4*-expressing *PGawB* P-element (Brand and Perrimon, 1993). To induce the exchange, both P-elements and a source of transposase were combined. We individually out-crossed 256 flies with potential exchanges to a *UAS-GFP* line and assayed the progeny for GFP expression in the dorsal half of the third instar eye disc. We recovered three independent exchanges in which the *LacZ*-expressing P-element was replaced by the *Gal4*-expressing P-element, thus producing three Dorsal-Eye Gal4 lines. These lines appear to behave identically, so we characterized one of them in more detail and refer to it simply as *DE-Gal4*.

The expression pattern of *DE-Gal4* in larvae was assessed by crossing *DE-Gal4* to a *UAS-LacZ* line and assaying β -galactosidase activity (Fig. 1a–e) or β -gal protein by immunostaining (Fig. 1f). In third instar eye imaginal discs, β -gal activity was restricted to

the dorsal region (Fig. 1a). A similar demarcation was evident in the antennal disc (Fig. 1a). We also analyzed eye-antennal expression during the second instar stage and found β -gal activity restricted to the dorsal area (Fig. 1b). In third instar wing discs, reporter activity was observed in the presumptive notum, ventral pleura, ventral hinge, and in the majority of the anterior wing pouch (Fig. 1c). β -gal activity in the haltere imaginal disc resembled the wing disc except that the haltere showed activity in nearly the entire pouch (Fig. 1d). Little, if any reporter activity was detected in leg imaginal discs (Fig. 1e). These imaginal disc expression patterns were similar to those reported for *mirror* (Kehl *et al.*, 1998). Likewise in embryos, β -gal staining resembled the expression pattern of *mirror* (McNeill *et al.*, 1997). Stage 11 embryos, for example, displayed staining in a repeating, segmental pattern as well as in the mesoderm (Fig. 1f).

To test the utility of *DE-Gal4* as a tool we performed a series of functional tests. First, we tested whether *DEGal4* can be used to induce phenotypes in the adult eye. We thus crossed *DE-Gal4* with a line expressing an RNAi construct targeting *eyes absent* (*eya*) mRNA. *Eya* is required for normal eye development and *eya* mutant flies have no eyes. Strikingly, *DE-Gal4* driven *eya-RNAi* expression resulted in flies with deleted dorsal eyes (Fig. 2b, control eye Fig. 2a). The *DE-Gal4* line can thus be used to induce adult phenotypes specifically in the dorsal eye. We note, however, that driving several other UAS transgenes driving the overexpression of genes (such as *UAS-yorkie* (*yki*), *UAS-bantam*, *UAS-Notch^{Intra}*), or RNAi targeting genes (such as *sine oculis*) was lethal in the embryo or pupal stage (for *yki* and *bantam* for example, see below for disc phenotypes).

We then tested whether *DE-Gal4* can be used to activate a signaling pathway readout specifically in the dorsal half of the eye disc. To this end we used *DE-Gal4* to drive the expression of *UAS-yorkie* (*yki*), a transcriptional coactivator in the Hippo growth control pathway and assayed the levels of the Hippo pathway readout *expanded-LacZ* (*ex-LacZ*). A previous study has shown that expression of *UAS-yki* induces *ex-LacZ* and causes overgrowth (Zhang *et al.*, 2008). In control discs lacking *DE-Gal4* and *UAS-yki*, β -galactosidase levels were similar in the dorsal and ventral halves of the discs (Fig. 2c). Conversely, *DE-Gal4*-driven *UAS-yki* induced high levels of *ex-LacZ* expression dorsally (Fig. 2d). In addition, these discs displayed dorsal-specific overgrowth. This experiment reveals that *DE-Gal4* can be used to activate pathway reporters specifically in the dorsal half of the developing eye.

Next, we asked if *DE-Gal4*-driven Flipase (Flp) could induce mitotic clones specifically in the dorsal half of the eye imaginal disc. In control discs, where Flp was expressed in all eye precursor cells by the *eyeless* promoter (*eyflp*), clones did not display obvious dorsoventral differences (Fig. 2e). In contrast, *DE-Gal4*-driven *UAS-Flp* induced clones almost exclusively in the dorsal half of the developing eye (Fig. 2f). Some discs displayed a few ventral clones (Fig. 2g), indicating that *DE-Gal4* has activity in the ventral half of the eye, albeit at a much lower level than in the dorsal half. We conclude that *DE-Gal4* can be used to generate mitotic clones almost exclusively in the dorsal half of the developing eye.

To investigate the extent of *DE-Gal4* expression in the ventral eye, we performed lineage tracing analysis using a “*flip-out-LacZ*” strategy (Struhl and Basler, 1993). In this method, Gal4-driven *UAS-Flp* induces recombination in the *flip-out-LacZ* cassette, leading to expression of *LacZ* in a cell and its descendants. Flies containing *DE-Gal4*, *UAS-Flp* (Duffy *et al.*, 1998), and *flip-out-LacZ* thus allowed us to determine the proportion of ventral eye cells that had ever expressed Gal4. In contrast to *DE-Gal4*, *UAS-LacZ* eye discs, which displayed staining exclusively in the dorsal half (Fig. 1a), *DE-Gal4*, *UAS-Flp*, *flip-out LacZ* eye discs displayed β -gal activity ubiquitously (Fig. 2h). This indicates that *DE-Gal4* drives some expression of *UAS-Flp* in the progenitors of the ventral cells. In contrast to the

recombination in the *flip-out-LacZ* cassette, however, the levels of this ventral *Flp* expression was not sufficient to efficiently cause the generation of inter-chromosomal recombination which was nearly exclusive to the dorsal compartment as discussed above (Fig. 2f,g). This difference is probably due to inter-chromosomal recombination requiring higher levels of *Flp* than the much more sensitive *flip-out* cassette. Altogether, these data thus indicate that *DE-Gal4* is expressed at low levels in the entire eye primordium at an early stage.

As a final functional test, we asked whether *DE-Gal4* could be used in combination with the *Flp-FRT* system to concomitantly generate control and experimental clones in a single eye disc. Clones of cells mutant for *yki*, a growth promoter, are very small in wing and eye discs (Fig. 2i) (Huang *et al.*, 2005). Expression of the *bantam* growth-promoting microRNA (Brennecke *et al.*, 2003) can significantly rescue the growth defects of *yki* mutant clones in the wing (Nolo *et al.*, 2006; Thompson and Cohen, 2006). However, it is unknown whether *bantam* can likewise rescue *yki* clones in the eye. Thus we used *DE-Gal4* to address this question. We generated larvae whose eye imaginal discs contained *yki*-mutant clones throughout and expressed *bantam* in the dorsal compartment by *DE-Gal4*. In such discs, ventral clones were indeed very small, however, dorsal clones displayed dramatic growth rescue (Fig. 2j). This demonstrates that *bantam* can rescue the growth of *yki* mutant clones in the developing eye. We conclude that *DE-Gal4* is useful for this type of approach because it allows the generation of experimental and control tissues in the same eye disc.

Our functional experiments demonstrate that *DE-Gal4* can be used to differentially manipulate cells in half of the developing eye, allowing multiple genotypes to be compared in a single eye imaginal disc. This type of genetic comparison approach is proving to be valuable in tumorigenesis research, where multiple genetic alterations, or synthetic phenotypes are required to achieve tumors (*RasV12*¹, *scrib*⁻ (Brumby and Richardson, 2003; Pagliarini and Xu, 2003)). Tools such as *DE-Gal4* could provide a means to perform efficient clone-based synthetic phenotype screens in vivo.

METHODS

P-Exchange

The *mirr*^{B1-12} *P{lacW}* insertion was replaced with a *P{GawB}* (Brand and Perrimon, 1993) *Gal4* enhancer trap P-element by generating females with both P-elements and $\Delta 2-3$ transposase (Sepp and Auld, 1999). These females were outcrossed to remove the $\Delta 2-3$ and the original *P{GawB}* chromosome. Progeny were screened for a change in eye color compared to the original *mirr*^{B1-12} line and 256 individual flies were crossed to a *UAS-GFP* line. Third instar larvae were dissected from each cross and assayed for GFP expression in the dorsal half of the eye disc. Three independent lines with the desired staining were isolated.

Betagalactosidase Antibody and Activity Staining

Imaginal disc β -gal activity staining was performed as described (Hiromi *et al.*, 1985). Embryo immunostaining was performed as described (Halder *et al.*, 1998).

Genetics

DE-Gal4 was crossed to *UAS-LacZ* to assay *Gal4* expression in imaginal discs and embryos. Lineage tracing was performed by combining *DE-Gal4*, *UAS-Flp* (Duffy *et al.*, 1998), and a *flip-out-LacZ* construct (Struhl and Basler, 1993) (*UAS-Flp*/+; +/+; *DE-Gal4*/ *P{Act5C(FRT.polyA)lacZ.nls1}*). Mitotic clones were generated with either *eyflp* (Newsome *et al.*, 2000) or *DE-Gal4* driving *UAS-Flp* and *FRT42DubiGFP*. In the *yki* mutant clone

rescue experiment, *yki eyflp* clones were generated on a background in which *UAS-bantamA* (Brennecke *et al.*, 2003) was driven by *DE-Gal4*.

Acknowledgments

The authors thank Robert Duncan for financially supporting this project. The authors also thank Kathleen Gajewski, Steve Cohen, Kwang-Wook Choi, Justin Kumar, Konrad Basler, and the Bloomington *Drosophila* Stock Center for providing fly stocks. The authors also thank Kathleen Gajewski and Chunyao Tao for technical assistance, as well as the other members of the Halder lab for helpful discussions.

Contract grant sponsors: National Institutes of Health grant (to G.H.) and a National Eye Institute training grant (to C.M.M.)

LITERATURE CITED

- Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. 1993; 118:401–415. [PubMed: 8223268]
- Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. *Bantam* encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene *hid* in *Drosophila*. *Cell*. 2003; 113:25–36. [PubMed: 12679032]
- Brumby AM, Richardson HE. *Scribble* mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in *Drosophila*. *EMBO J*. 2003; 22:5769–5779. [PubMed: 14592975]
- Calleja M, Moreno E, Pelaz S, Morata G. Visualization of gene expression in living adult *Drosophila*. *Science*. 1996; 274:252–255. [PubMed: 8824191]
- Cavodeassi F, Diez Del Corral R, Campuzano S, Dominguez M. Compartments and organising boundaries in the *Drosophila* eye: The role of the homeodomain Iroquois proteins. *Development*. 1999; 126:4933–4942. [PubMed: 10529412]
- Cavodeassi F, Modolell J, Gomez-Skarmeta JL. The Iroquois family of genes: From body building to neural patterning. *Development*. 2001; 128:2847–2855. [PubMed: 11532909]
- Choi KW, Mozer B, Benzer S. Independent determination of symmetry and polarity in the *Drosophila* eye. *Proc Natl Acad Sci USA*. 1996; 93:5737–5741. [PubMed: 8650162]
- Crocker JA, Ziegenhorn SL, Holmgren RA. Regulation of the *Drosophila* transcription factor. Cubitus interruptus, by two conserved domains. *Dev Biol*. 2006; 291:368–381. [PubMed: 16413529]
- Duffy JB, Harrison DA, Perrimon N. Identifying loci required for follicular patterning using directed mosaics. *Development*. 1998; 125:2263–2271. [PubMed: 9584125]
- Gomez-Skarmeta JL, Diez del Corral R, de la Calle-Mustienes E, Ferre-Marco D, Modolell J. Araucan and caupolican, two members of the novel *iroquois* complex, encode homeoproteins that control proneural and vein-forming genes. *Cell*. 1996; 85:95–105. [PubMed: 8620542]
- Halder G, Callaerts P, Flister S, Walldorf U, Kloter U, Gehring WJ. Eyeless initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. *Development*. 1998; 125:2181–2191. [PubMed: 9584118]
- Harrison DA, Binari R, Nahreini TS, Gilman M, Perrimon N. Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *Embo J*. 1995; 14:2857–2865. [PubMed: 7796812]
- Hay BA, Wolff T, Rubin GM. Expression of baculovirus P35 prevents cell death in *Drosophila*. *Development*. 1994; 120:2121–2129. [PubMed: 7925015]
- Hazelett DJ, Bourouis M, Walldorf U, Treisman JE. *Decapentaplegic* and *wingless* are regulated by *eyes absent* and *eyegone* and interact to direct the pattern of retinal differentiation in the eye disc. *Development*. 1998; 125:3741–3751. [PubMed: 9716539]
- Hiromi Y, Kuroiwa A, Gehring WJ. Control elements of the *Drosophila* segmentation gene *fushi tarazu*. *Cell*. 1985; 43:603–613. [PubMed: 3935327]
- Huang J, Wu S, Barrera J, Matthews K, Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the *Drosophila* homolog of YAP. *Cell*. 2005; 122:421–434. [PubMed: 16096061]

- Kehl BT, Cho KO, Choi KW. *Mirror*, a *Drosophila* homeobox gene in the *Iroquois* complex, is required for sensory organ and alula formation. *Development*. 1998; 125:1217–1227. [PubMed: 9477320]
- Kos CH. Cre/loxP system for generating tissue-specific knockout mouse models. *Nutr Rev*. 2004; 62:243–246. [PubMed: 15291397]
- McNeill H, Yang CH, Brodsky M, Ungos J, Simon MA. *Mirror* encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the *Drosophila* eye. *Genes Dev*. 1997; 11:1073–1082. [PubMed: 9136934]
- Netter S, Fauvarque MO, Diez del Corral R, Dura JM, Coen D. White+ transgene insertions presenting a dorsal/ventral pattern define a single cluster of homeobox genes that is silenced by the polycomb-group proteins in *Drosophila melanogaster*. *Genetics*. 1998; 149:257–275. [PubMed: 9584101]
- Newsome TP, Asling B, Dickson BJ. Analysis of *Drosophila* photo-receptor axon guidance in eye-specific mosaics. *Development*. 2000; 127:851–860. [PubMed: 10648243]
- Nolo R, Morrison CM, Tao C, Zhang X, Halder G. The *bantam* microRNA is a target of the hippo tumor-suppressor pathway. *Curr Biol*. 2006; 16:1895–1904. [PubMed: 16949821]
- Pagliarini RA, Xu T. A genetic screen in *Drosophila* for metastatic behavior. *Science*. 2003; 302:1227–1231. [PubMed: 14551319]
- Prober DA, Edgar BA. Ras1 promotes cellular growth in the *Drosophila* wing. *Cell*. 2000; 100:435–446. [PubMed: 10693760]
- Sepp KJ, Auld VJ. Conversion of lacZ enhancer trap lines to GAL4 lines using targeted transposition in *Drosophila melanogaster*. *Genetics*. 1999; 151:1093–1101. [PubMed: 10049925]
- Struhl G, Basler K. Organizing activity of wingless protein in *Drosophila*. *Cell*. 1993; 72:527–540. [PubMed: 8440019]
- Tanimoto H, Itoh S, ten Dijke P, Tabata T. Hedgehog creates a gradient of DPP activity in *Drosophila* wing imaginal discs. *Mol Cell*. 2000; 5:59–71. [PubMed: 10678169]
- Thompson BJ, Cohen SM. The Hippo pathway regulates the *bantam* microRNA to control cell proliferation and apoptosis in *Drosophila*. *Cell*. 2006; 126:767–774. [PubMed: 16923395]
- Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*. 1993; 117:1223–1237. [PubMed: 8404527]
- Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J. The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. *Dev Cell*. 2008; 14:377–387. [PubMed: 18258485]

FIG. 1.

DE-Gal4 expression pattern assayed by *UAS-LacZ* expression. *DE-Gal4* was crossed to *UAS-LacZ*. (a–f) β -galactosidase activity staining in imaginal discs. (a) A third instar eye-antennal imaginal disc displaying dorsally-restricted β -gal activity. (b) A second instar eye-antennal disc likewise showed dorsally-restricted staining. (c) A wing disc and (d) a haltere disc displaying staining in the presumptive nota, pleura, and pouch regions. (e) A leg disc showing little, if any staining. (f) β -gal immunostaining in a stage 11 embryo was detected in the mesoderm and in a segmentally repeating pattern. In all images, anterior is left and dorsal is up.

FIG. 2.

DE-Gal4 lineage tracing and functional analysis. (a) Eye of a *DE-Gal4* fly. The dorsal part expresses higher levels of the *white*⁺ minigene that is part of the P-element harboring the *Gal4* sequence. (b) Eye of a fly expressing RNAi targeting the *eya* gene driven by *DE-Gal4*. The dorsal part of the eye is ablated as the dorsal, darker part of the eye is missing compared to (a). Images (c–j) depict third instar eye imaginal discs. (c) A disc bearing an *expanded-LacZ* reporter construct immunostained for β -gal showed similar staining in the dorsal and ventral halves. (d) An *expanded-LacZ* disc expressing *UAS-yki* under the control of *DE-Gal4* and immunostained for β -gal. Dorsal staining was significantly stronger. (e) A disc with *eyflp*-induced mitotic clones did not display an obvious dorsoventral difference. (f, g) Discs in which *DE-Gal4*-driven *UAS-Flp* was used to induce mitotic clones. Clones were

predominantly generated in the dorsal compartment. (h) A disc in which *DE-Gal4*-driven *UAS-Flp* induced recombination in a “*flip-out LacZ*” transgene, thus expressing LacZ. β -gal activity was detected in nearly all cells of the eye-antennal disc. (i) A disc with *eyflp*-generated clones of a *yki*^{B5} mutant chromosome. (j) A disc containing *eyflp*-generated *yki*^{B5} clones and expressing *UAS-bantamA* in the dorsal compartment by *DE-Gal4*.