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A broad expression profile of the *GMR*-GAL4 driver in *Drosophila melanogaster*

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ABSTRACT. The GAL4/*UAS* binary system has been widely used in *Drosophila melanogaster* for ectopic expression of transgenes in a tissue-specific manner. The *GMR*-GAL4 driver, which expresses the yeast transcription factor GAL4 under the control of glass multiple reporter (*GMR*) promoter elements, has been commonly utilized to express target transgenes, specifically in the developing eye. However, we have observed abnormal wing phenotypes; this is a result of the activity of critical wing developing genes, e.g., components of the Notch or Wg pathway, that are up- or down-regulated under the control of the *GMR*-GAL4 driver. X-gal staining confirmed that *UAS*-LacZ is expressed in third-instar larva wing imaginal discs, as well as in eye discs, when driven by the *GMR*-GAL4 driver. Furthermore, we found that *GMR*-GAL4 also drives *UAS*-LacZ expression in other tissues, such as brain, trachea, and leg discs. These results indicate that *GMR*-GAL4 has a broad expression profile, rather than the eye-specific pattern described previously, and that one should be careful when using it as a tool for targeted gene expression.

Key words: *GMR*-GAL4; *Drosophila*; Wing; Expression

INTRODUCTION

The GAL4/*UAS* bipartite expression system has been utilized as an extremely powerful tool to ectopically express transgenes in specific patterns in *Drosophila melanogaster* (Duffy, 2002; Traven et al., 2006). In this system, the yeast transcription factor GAL4 is driven by various promoter sequences, and thus, exhibits many different temporal and spatial expression profiles (Laughon et al., 1984; Brand and Perrimon, 1993). On the other hand, the transgenes are placed under the control of the GAL4 target sequence, upstream activation sequence (*UAS*), and could be transcriptionally activated by GAL4 (Brand and Perrimon, 1993). *GMR*-GAL4 was constructed by Freeman in 1996 and was reported to drive the expression of target genes in all cells posterior to the morphogenetic furrow in the developing eye specifically (Song et al., 2000). Since then, many overexpression studies have been carried out in *Drosophila*, using the *GMR*-GAL4 driver line with attention being focused on the developing eyes.

The Notch signaling pathway, which has been highly conserved in most multi-cellular organisms, is important for cell-cell communication (Major and Irvine, 2005). *Notch* was first identified in *Drosophila* as an important gene for wing development (de Celis and Garcia-Bellido, 1994). It is required for the establishment of the dorsal-ventral compartment border, and the proper development of wing margin, veins, and sensory organs (Casso et al., 2011). The Notch receptor is activated by the Delta and Jagged/Serrate families of membrane-bound ligands (Weinmaster, 1997).

The Wnt signaling pathway is another evolutionary conserved pathway that plays pivotal roles in embryogenesis and other physiological processes. Dysfunction of the Wnt pathway leads to many diseases including cancer (Lie et al., 2005). The *Drosophila wingless* gene (*wg*) is the founding member of the Wnt family, which was originally identified as a recessive mutation affecting wing and haltere development (Sharma and Chopra, 1976). The Wg protein can bind to cell-surface receptors of the Frizzled family, which activates Dishevelled (Dsh), ultimately resulting in the accumulation of Armadillo (Arm), which reaches the nucleus and subsequently promotes specific gene expression (Benitez et al., 2009).

In this report, we showed that expression of wild-type, dominant negative or RNAi of the critical wing developing genes, e.g., Notch and Wg pathway components, under the control of three independent *GMR*-GAL4 drivers, results in various abnormal wing phenotypes, suggesting that *GMR*-GAL4 is expressed in the developing wing. We performed X-Gal staining to examine *UAS*-LacZ expression driven by *GMR*-GAL4, and confirmed that *GMR*-GAL4 is expressed in third-instar larval wing discs, as well as in other tissues including brain, trachea and leg discs. These data demonstrate that *GMR*-GAL4 has a much broader expression pattern than previously described, which should be borne in mind when over-expression analysis is performed using *GMR*-GAL4 as the driver.

MATERIAL AND METHODS

Drosophila strains

GMR-GAL4, *UAS*-LacZ, *UAS*-Dl, *UAS*-N.RNAi, *UAS*-Dl^{DN}, *UAS*-Wg, *UAS*-Dsh were obtained from the Bloomington *Drosophila* Stock Center and *UAS*-wg.RNAi from the VDRC Center.

X-Gal staining

Eye and wing discs were dissected from 3rd-instar larvae in PBST and stained for β-galactosidase activity as previously described (Xue et al., 2007).

RESULTS AND DISCUSSION

The *GMR*-GAL4 driver has been commonly used to induce ectopic expression of target genes in the developing eyes, more specifically, in the cells posterior to the morphogenetic furrow in the eye discs (Freeman, 1996). The expression profile of *GMR*-Gal4 in other tissues, however, has not been well characterized. To address this issue, we expressed Delta (Dl), a ligand of the Notch signaling that plays important roles in the development of most tissues, under the control of *GMR*-GAL4 (*GMR*>Dl). Three independent *GMR*-Gal4 lines were tested in this and all subsequent experiments and similar results were obtained.

We found the *GMR*>Dl flies not only show the eye phenotype as previously reported (Shalaby et al., 2009; data not shown), but also display a wing phenotype. In most cases, the distal part of vein L5 was lost in the *GMR*>Dl wings (Figure 1C and D), as compared to the wild-type control (Figure 1A and B). Since Notch signaling is known to inhibit vein formation and regulate dorsal-ventral patterning in wing development, our finding suggests that *GMR*-Gal4 might be expressed in the developing wings. Consistent with this explanation, downregulation of the Notch pathway by the expression of a Notch RNAi under the control of *GMR*-Gal4 resulted in extra veins (Figure 1E and F), and expression of a dominant negative form of Notch generated thicker veins (Figure 1G and H), and sometimes water bubbles between dorsal/ventral layers in the wing (Figure 1J, compared to wild type in 1I).

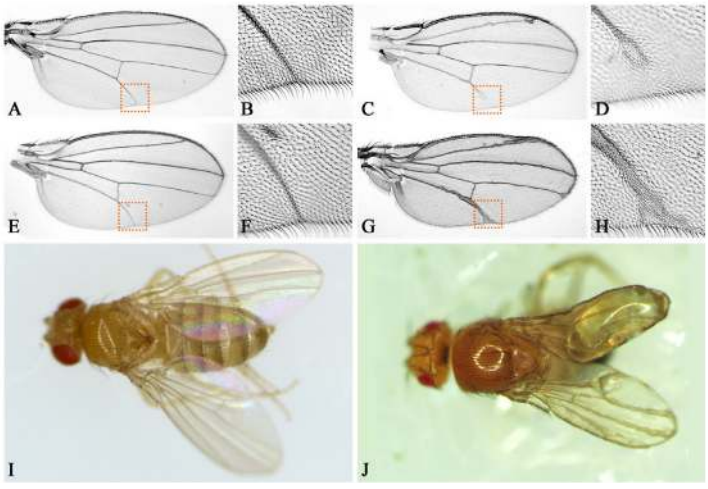

Figure 1. Various wing phenotypes resulting from expression of Notch pathway components driven by *GMR*-GAL4. Compared to a wild-type wing (**A**, **B** and **I**), activation of Notch signaling by *GMR*-Gal4 inhibits vein L5 formation in the distal area (**C** and **D**, *GMR*-Gal4/*UAS*-Dl), while downregulation of Notch signaling by *GMR*-Gal4 results in extra veins (**E** and **F**, *GMR*-Gal4/*UAS*-N.RNAi), thicker veins (**G** and **H**, *GMR*-Gal4/*UAS*-Dl^{DN}), and water bubbles between dorsal/ventral layers (**J**, *GMR*-Gal4/*UAS*-N^{DN}). B, D, F, and H are magnifications of the boxed areas in A, C, E, and G, respectively.

The Wingless (Wg) signaling pathway is another crucial regulator of *Drosophila* wing development. While ectopic Wg signaling results in extra vein and bristle formation, insufficient Wg signaling leads to loss of wing tissue (Williams et al., 1993). We found that downregulation of Wg signaling by the expression of a wg.RNAi under the control of *GMR*-Gal4 results in loss of wing margin tissues (Figure 2C and D), and expression of its downstream component Arm RNAi leads to shrunken wings (Figure 2B, compare to wild type in 2A). In addition, ectopic expression of Wg or its downstream component Dsh driven by *GMR*-Gal4 produced extra veins (Figure 2E and F) and bristles (Figure 2G and H).

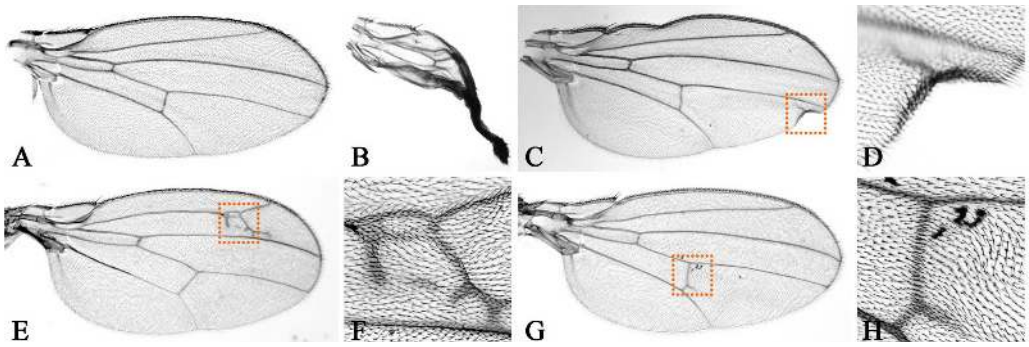

Figure 2. Various wing phenotypes resulting from expression of Wg pathway components driven by *GMR*-GAL4. Compared to a wild-type wing (**A**), downregulation of Wg signaling by *GMR*-Gal4 results in shrunken wing (**B**, *GMR*-Gal4/*UAS*-Arm.RNAi), or notch of wing margin (**C** and **D**, *GMR*-Gal4/*UAS*-Wg.RNAi), while activation of Wg signaling by *GMR*-Gal4 induces the formation of extra veins (**E** and **F**, *GMR*-Gal4/*UAS*-Wg) or bristles (**G** and **H**, *GMR*-Gal4/*UAS*-Dsh). D, F and H are magnifications of the boxed areas in C, E and G, respectively.

The adult wing phenotypes described above suggest that *GMR*-Gal4 is most likely also expressed in the developing wing. To confirm this notion, we crossed three independent *GMR*-Gal4 lines to *UAS*-LacZ and examined the expression pattern of LacZ in 3rd-instar larval wing discs. Compared to the wild-type control (Figure 3A), all three *GMR*-Gal4 lines were able to drive LacZ expression in the wing discs, albeit at different strength (Figure 3B-D). The expression patterns of three *GMR*-Gal4 lines were partially, but not completely overlapping (Figure 3B-D). As a positive control, LacZ was induced in the eye imaginal discs, most prominently, posterior to the morphogenetic furrow, by all three *GMR*-Gal4 drivers (Figure 3F-H).

We further investigated the expression pattern of *GMR*-Gal4 in other tissues, and found LacZ expression in the larval brain (Figure 4D), leg disc (Figure 4E) and trachea (Figure 4F), as compared to the control (Figure 4A-C). Taken together, our results indicate that *GMR*-Gal4 has a much broader expression profile, rather than the eye-specific pattern, as commonly regarded. Researchers should be cautious in interpreting certain phenotypes, such as lethality and behavioral defects, resulting from transgene over-expression driven by the *GMR*-Gal4 driver.

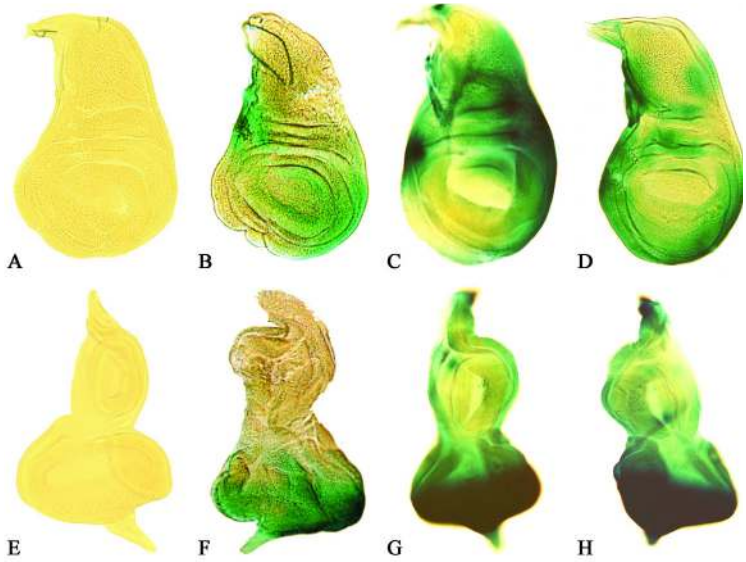

Figure 3. *GMR*-GAL4 is expressed in wing and eye discs. X-Gal staining of a *UAS*-LacZ reporter gene driven by no (**A** and **E**) or three independent *GMR*-GAL4 drivers (**B-D**, **F-H**) in 3rd-instar larval wing (**A-D**) or eye (**E-H**) discs.

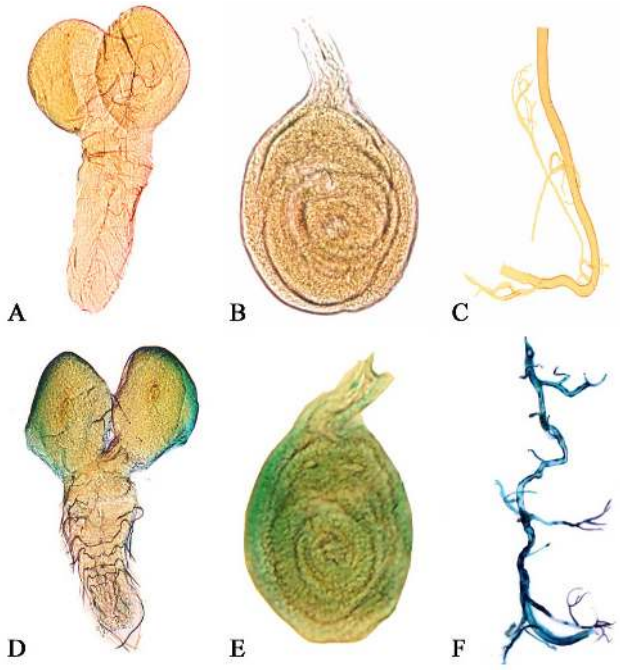

Figure 4. *GMR*-Gal4 is expressed in other developing tissues. X-Gal staining of a *UAS*-LacZ reporter gene driven by no (**A-C**) or a *GMR*-GAL4 driver (**D** and **E**) in 3rd-instar larval brain (**A** and **D**), leg disc (**B** and **E**) or trachea (**C** and **F**).

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