RESEARCH ARTICLE

The commonly used eye-specific *sev-GAL4* and *GMR-GAL4* drivers in *Drosophila melanogaster* are expressed in tissues other than eyes also

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Abstract

The binary *GAL4-UAS* system of conditional gene expression is widely used by *Drosophila* geneticists to target expression of the desired transgene in tissue of interest. In many studies, a preferred target tissue is the *Drosophila* eye, for which the *sev-GAL4* and *GMR-GAL4* drivers are most widely used since they are believed to be expressed exclusively in the developing eye cells. However, several reports have noted lethality following expression of certain transgenes under these GAL4 drivers notwithstanding the fact that eye is not essential for survival of the fly. Therefore, to explore the possibility that these drivers may also be active in tissues other than eye, we examined the expression of *UAS-GFP* reporter driven by the *sev-GAL4* or *GMR-GAL4* drivers. We found that both these drivers are indeed expressed in additional tissues, including a common set of specific neuronal cells in larval and pupal ventral and cerebral ganglia. Neither *sev* nor *glass* gene has so far been reported to be expressed in these neuronal cells. Expression pattern of *sev-GAL4* driver parallels that of the endogenous Sevenless protein. In addition to cells in which *sev-GAL4* is expressed, the *GMR-GAL4* is expressed in several other larval cell types also. Further, two different *GMR-GAL4* lines also show some specific differences in their expression domains outside the eye discs. These findings emphasize the need for a careful confirmation of the expression domains of a GAL4 driver being used in a given study, rather than relying only on the empirically claimed expression domains.

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Introduction

The binary *GAL4-UAS* system (Brand and Perrimon 1993) has greatly facilitated functional genomics through targeted expression of the desired transgene in a spatially and temporally regulated manner in *Drosophila melanogaster*. One of the great advantages of this system is that it permits ectopic expression of potentially harmful/toxic transgenes in specific tissues which are not essential for survival. *Drosophila* eye is one such organ which has been widely used as a model for studying genetic interaction between different genes, especially in relation to neurogenetic studies. The most widely used *GAL4* drivers for targeting the *UAS*-carrying responder gene's expression to developing eyes are the *GMR-GAL4* and *sevenless-GAL4* transgenes. The *GMR-GAL4* (Freeman 1996) driver is believed to be expressed from mid-third-instar stage through pupal development exclusively in all cells posterior to the morphogenetic furrow (MF) in differentiating larval eye discs (Freeman 1996). On the other hand, following the reported expression domain of the *sevenless* (*sev*) gene (Bowtell *et al.* 1991; Maxiner *et al.* 1998; Firth *et al.* 2006), the *sev-GAL4* driver is believed to be expressed exclusively in the developing photoreceptor cells, initially in the nine Sevenless equivalence group cells in each ommatidial cluster (Tomlinson *et al.* 1987), which includes the precursors of the R1/R6, R3/R4, R7 photoreceptors and four cone cells, and finally only in the R7 photoreceptors. These two drivers have been used in a very large number of studies with the belief that expression of the target UAS-transgene would be driven only in the eye discs/eyes as noted above. However, several studies (Firth *et al.* 2006; Morris *et al.* 2006; Mallik and Lakhotia 2009a, b) have reported pupal lethality following *GMR-GAL4* or *sev-GAL4* driven expression of certain UAS-transgene constructs, notwithstanding the fact that even a complete absence of eyes has no effect on viability of pupae and emergence of adult flies (Sang and Jackson 2005). These observations raise the possibility that these two drivers activate expression of the target transgene in some other tissues also, which may actually be responsible for the observed lethality. Therefore, we examined *sev-GAL4* or *GMR-GAL4* driven expression of the *UAS-GFP* reporter transgene to identify the embryonic, larval and pupal tissues

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in which these drivers are active. Our results showed that, in addition to the above noted well-known expression domains of these two GAL4 drivers in developing eyes, each of them specifically activates the reporter gene in several other cells as well, including specific neuronal cells in larval and pupal brain and the ventral ganglia.

Materials and methods

Fly stocks

All flies stocks and crosses were maintained on standard agar cornmeal medium at 24 ± 1°C. The w^{1118}; *sev-GAL4*; + (no. 5793; Bailey 1999), w^{1118}; *UAS-GFP*; + (no. 1521), and w^{1118}; *Ddc-GAL4*; + (no. 7010), stocks were obtained from the Bloomington Stock Center (USA). Two *GMR-GAL4* stocks, one with the G*MR-GAL4* transgene (Freeman 1996) inserted on chromosome 2 (w^{1118}; *GMR-GAL4*; +) and the other with the G*MR-GAL4* transgene inserted on chromosome 3 (w^{1118}; +; *GMR-GAL4*, Igaki *et al.* 2002) were used. These two transgene insertions are designated in the following as *GMR-GAL4*^{2} and *GMR-GAL4*^{3}, respectively.

Reporter gene expression

The w^{1118}; *GMR-GAL4*^{2}; +, w^{1118}; +; *GMR-GAL4*^{3} or w^{1118}; *sev-GAL4*; + flies were crossed with w^{1118}; *UAS-GFP*; + flies and the w^{1118}; *sev-GAL4/UAS-GFP*, w^{1118}; *GMR-GAL4*^{2}/*UAS-GFP* or w^{1118}; *UAS-GFP/+*; *GMR-GAL4*^{3}/+ progeny eggs were collected at hourly intervals. Larvae that hatched during a period of 1 h were separated to obtain synchronously growing larvae. Similarly, larvae that began pupation during a period of 1 h were separated to obtain pupae of defined age (expressed as h after pupa formation or h APF). The first, second and late third instar actively moving larvae and 8–9 a and 24–25 h APF pupae were dissected in Poels' salt solution (PSS, Lakhotia and Tapadia 1998) and internal tissues fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, 130 mm NaCl, 7 mm Na_2HPO_4, 3 mm KH_2PO_4, pH 7.2) for 20 min. After three 10 min washes in 0.1% PBST (PBS + 0.1% Triton-X-100), the tissues were counterstained with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride, 1 μg/mL) and mounted in DABCO for imaging the GFP expression. To examine the expression of the reporter gene in embryonic stages, embryos of different genotypes were fixed after removing chorion and vitelline membranes, and were immunostained using rabbit antiGFP primary (Sigma Aldrich, St Louis, USA, 1 : 50) and Alexa Flour 488 conjugated antirabbit secondary (Molecular Probes, Eugene, USA, 1 : 200) antibodies as described (Van Vactor and Kopczynski 1999). Chromatin was counterstained with DAPI (1 μg/mL) and the embryos were mounted in DABCO.

Nature and anatomical positions of the GFP expressing cells in the brain and ventral ganglia (central nervous system, CNS) of w^{1118}; *sev-GAL4/UAS-GFP*, w^{1118}; *UAS-GFP/+*; *GMR-GAL4*^{3}/+ and w^{1118}; *GMR-GAL4*^{2}*/UAS-GFP* larvae were ascertained by immunostaining with mouse antiElav (Developmental Studies Hybridoma Bank (DSHB), 1 : 100), or mouse antiFasciclin II (1D4, DSHB, 1 : 75) primary and antimouse Cy3 (Sigma Aldrich, 1 : 200) secondary antibodies as described earlier (Singh and Lakhotia 2012). The immunostained tissues were counterstained with DAPI (1 μg/mL) and mounted in DABCO for imaging.

To know the nature of CNS neurons expressing *UAS-GFP* under the *sev-GAL4* or *GMR-GAL4* drivers (see Results), dopaminergic (*Ddc-GAL4*) neuron-specific driver was used to drive the *UAS-GFP* reporter either alone or in conjunction with *sev-GAL4* driver in progeny of w^{1118}; *sev-GAL4 UAS-GFP*; + female and w^{1118}; *Ddc-GAL4*; + male flies. The GFP expression in CNS of the progeny larvae/pupae was examined as above. To determine if the *sev-GAL4>UAS-GFP* expressing neurons in the CNS were cholinergic, late third instar w^{1118}; *sev-GAL4/UAS-GFP* larval CNS were immunostained with anticholine acetyltransferase (DSHB, 1 : 100) primary and antimouse Cy3 (Sigma Aldrich, 1 : 200) secondary antibodies.

To know if the *sev-GAL4>UAS-GFP* expression is coincident with expression of the resident *sev* gene, CNS and eye discs of wandering late third instar (w^{1118}; *sev-GAL4/UAS-GFP*) larvae were immunostained with goat antiSevenless (dR-15, Santa Cruz, 1 : 50) and antiGoat Cy3 (Jackson Immuno, 1 : 200) antibodies. The tissues were counterstained with DAPI (1 μg/mL), mounted in DABCO and examined under Carl Zeiss 510 Meta laser scanning confocal microscope.

Results

The *sev-GAL4* or *GMR-GAL4* driven expression of *UAS-GFP* reporter transgene in the ommatidial units behind MF in developing late third instar and pupal eye discs is in agreement with the widely known patterns (figure 1, a&b). Both drivers led to GFP expression in the ommatidial units, optic nerve and axonal projections in the optic lobes of larval/pupal brain ganglia (figure 1, c&d). As expected (Bowtell *et al.* 1991; Freeman 1996), *GMR-GAL4* driven GFP expression was more extensive than that of the *sev-GAL4* driver (figure 1). *UAS-GFP* expression patterns driven in larval or pupal eye discs by *GMR-GAL4*^{2}or *GMR-GAL3*^{2} drivers were seen to be identical (not shown).

Expression of sev-GAL4 driver in other tissues

A detailed examination of GFP expression in embryos, and larval and pupal stages revealed that *sev-GAL4* drives *UAS-GFP* expression in tissues other than eye discs also (see table 1; figures 2–4). The most remarkable expression of *sev-GAL4* (figure 2) driver, other than the eye discs, was in CNS. A specific group of segmentally repeated dorsomedially placed bilateral pairs of cells (figure 2, a, c, e, g&i) in the ventral ganglia of all the three larval instars and early pupal stages expressed GFP when driven by *sev*-GAL4 driver. The GFP-positive cells in ventral ganglia and eye discs expressed

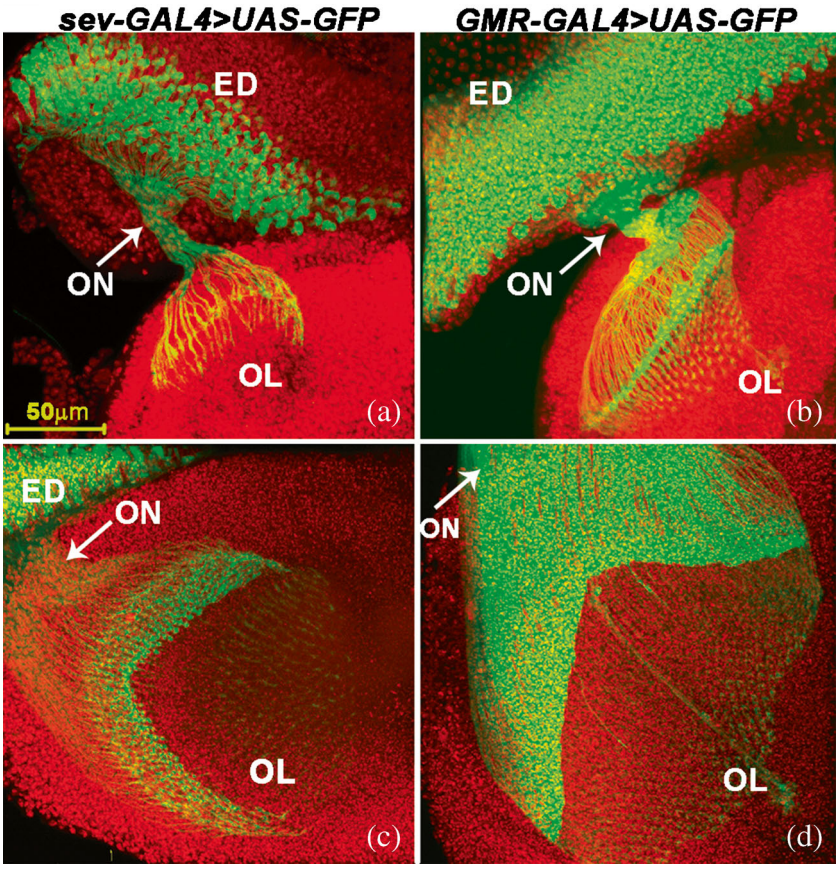

Figure 1. The *sev-GAL4* and *GMR-GAL4* drivers are active in eye discs and optic lobes during late larval and early pupal stages. Confocal projection images showing the widely known reporter *UAS-GFP* expression (green) driven by *sev-GAL4* (a, c) or *GMR-GAL4*^{2} (b, d) drivers in developing ommatidial units in eye discs (ED), optic nerve (ON) and axonal projections on the optic lobes (OL) of third instar larvae (a, b) and 8–9 h old pupae (c, d). Nuclei are couterstained with DAPI (red). Scale bar in (a) applies to (a–d).

Table 1. Summary of *sev-GAL4* or *GMR-GAL4* driven *UAS-GFP* reporter gene expression in embryonic, larval and pupal tissues.

	Genotypes		
Developmental stage	w^{1118}; *sev-GAL4/UAS-GFP* (figures 1 and 2)	w^{1118}; *GMR-GAL4*^{2}*/UAS-GFP* (figures 1, 6 and 7)	w^{1118}; *UAS-GFP/+*; *GMR-GAL4*^{3}*/+* (figures 6 and 7)
Embryo	No expression.	Bolwig's organ, eye primodia.	Same as *GMR-GAL4*^{2}, in addition, salivary glands.
First instar larvae	CNS: dorsomedial pairs of neurons in T3 to A7 neuromeres of ventral ganglia and several other scattered neuronal cells.	CNS: as in *sev-GAL4*. Additionally, Bolwig's organ and nerve, ocellar neurons and their axonal projections; clypeo-labral organ.	Same as *GMR-GAL4*^{2}; in addition, salivary glands.
Second instar larvae	As in 1st instar larvae.	As in 1st instar larvae.	As in 1st instar larvae.
Third instar larvae (late)	CNS: as in 1st instar larvae. Additionally, R3, R4, R7, R1 and R6 photoreceptor neurons in eye discs posterior to MF and their axons projecting on brain optic lobe.	CNS: as in *sev-GAL4*. All cells of differentiating ommatidial units posterior to MF and axonal projections on optic lobes; ocellar neurons and their projections. Additionally, spiracular glands; some tracheal cells.	As in *GMR-GAL4*^{2}; except (i) signal in tracheae is more extensive than with *GMR-GAL4*^{2} and (ii) spiracular glands do not show GFP signal. Late third instar salivary glands also do not show GFP signal. In addition, mushroom body and several other cells in brain ganglia.
8–9 h APF	As in third instar larvae.	As in third instar larvae.	As in third instar larvae.
24–25 h APF	Dorsomedial pair of neurons in T3, scattered neurons in ventral part of ventral ganglia; eye disc as in early pupae.	CNS and eye discs: as in *sev-GAL4*. Additionally, ocellar neurons and their projections on brain.	Same as *GMR-GAL4*^{2}.

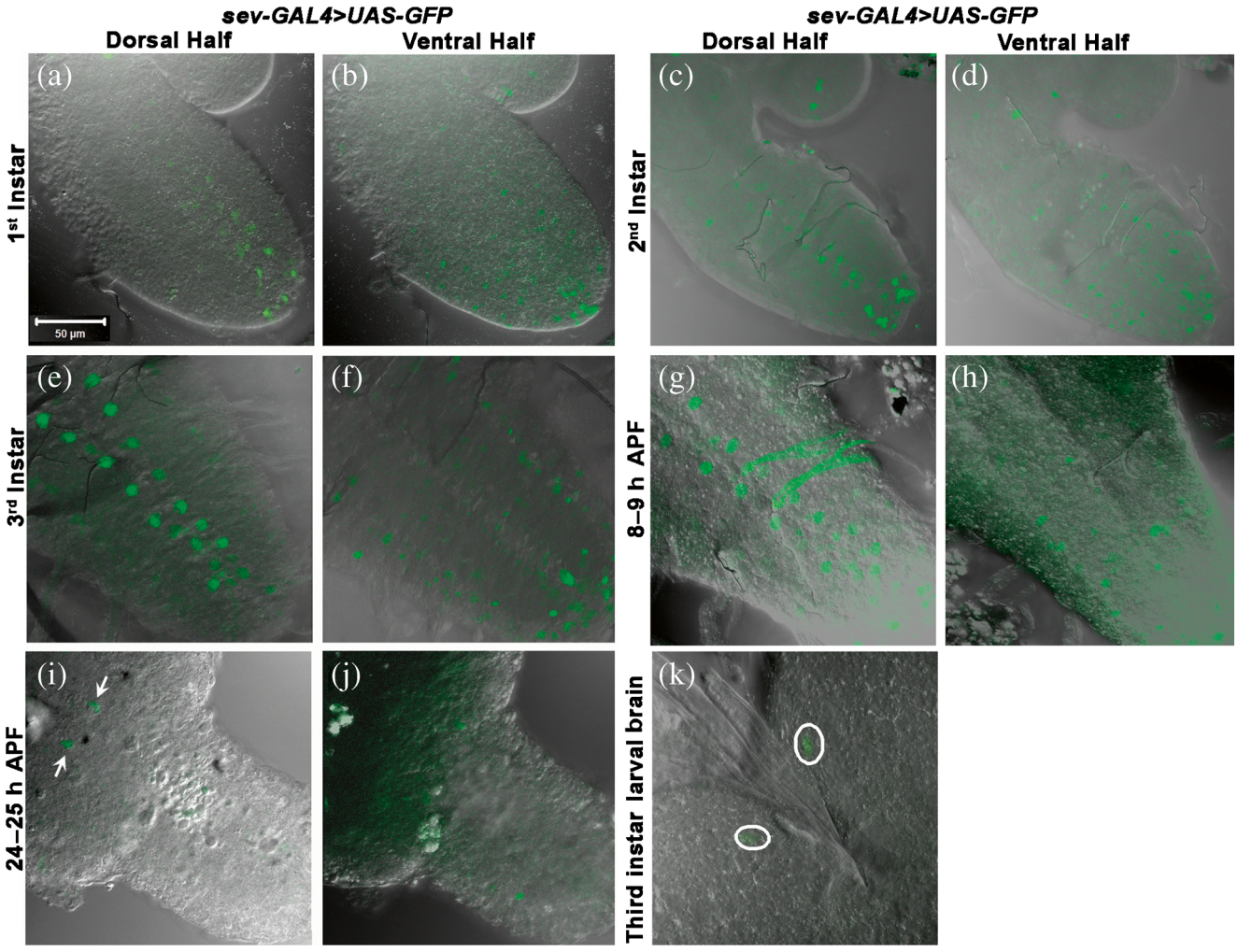

Figure 2. Expression of *sev-GAL4* driver in larval and pupal CNS. Confocal projections showing *sev-GAL4* (a–j) driven GFP expression (green), against DIC image, in ventral ganglia from different larval and pupal stages (indicated on left of each row). Projection images of dorsal (a, c, e, g and i) and ventral (b, d, f, h and j) sides of each ventral ganglia are shown separately. Arrows in (i) point to the persisting anterior most pair of dorsomedial GFP-expressing neurons. (k) shows a confocal projection image of the paired neurons (encircled by white line) on dorsal side of third instar larval brain ganglia which expresses *sev-GAL4* driven GFP (green). Scale bar in (a) applies to (a–k).

Elav as evidenced by immunostaining with Elav-antibody (figure 3a). Since, Elav protein specifically identifies neuronal cells (Robinow and White 1991), the *sev-GAL4* driven GFP expressing cells in eye discs and CNS are neuronal in nature.

The GFP expression in the segmentally placed dorsomedial pairs of neurons progressively waned by 24 h APF, leaving GFP expression only in the anterior-most pair (figure 2i). In addition to these paired neurons, the *sev-GAL4* driver also led to GFP expression in many other cells at the posterior end and in ventrolateral sides of larval and pupal ventral ganglia (figure 2, b, d, f, h&j). The *sev-GAL4* driver also activated *UAS-GFP* in a pair of dorsomedially placed neurons in the brain of third instar larvae (figure 2k).

Anatomical location of the dorsomedially placed pairs of GFP expressing neurons in ventral ganglia was ascertained by immunostaining of the *sev-GAL4>GFP* expressing pupal CNS with anti-Fasciclin II (Fas II), which delineates the neuromeres (Vomel and Wegener 2008). As shown in figure 3b, the last thoracic (T3) and each of the abdominal (A1–A7) neuromeres carried a dorsomedial pair of GFP-expressing neurons located close to anterior margins of the Fas II tracts. This pattern of location of the GFP-positive neurons in ventral ganglia is strongly reminiscent of the arrangement of RP2 motor neurons in ventral ganglia, which are also reported to disappear by 24 h APF stage (Winbush and Weeks 2011). This possibility of their being same needs to be further examined.

We did not find *sev-GAL4* driven GFP expression during embryonic stages or in any part of larval/pupal gut, fat body or other imaginal discs. Dopaminergic or cholinergic nature of the characteristically located segmental dorsomedial pairs of neurons in ventral ganglia was ascertained by coexpressing the *sev-GAL4* and *Ddc-GAL4* to drive GFP expression or by immunostaining of the *sev-GAL4>UAS-GFP* expressing CNS for cholinergic neurons (figure 4). Coexpression of

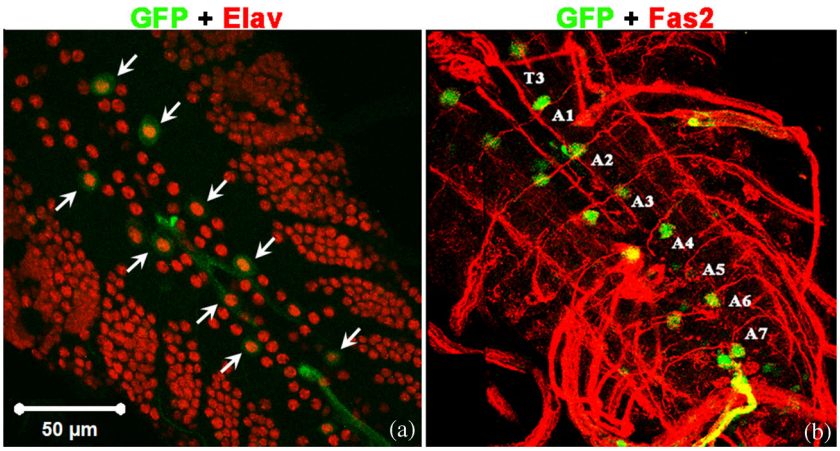

Figure 3. The *sev-GAL4* driven *UAS-GFP* expressing cells in ventral ganglia express Elav and are arranged in a neuromeric pattern. (a) Confocal projection image of anti-Elav (red) stained dorsal half of late larval ventral ganglia expressing *sev-GAL4>UAS-GFP* (green); some of the GFP expressing dorsomedial pairs of neurons are indicated by arrows. (b) Confocal projection image of anti-Fas II (red) stained dorsal half of early pupal ventral ganglia expressing *sev-GAL4>UAS-GFP* (green); the T3, A1–A7 neuromeres are marked. Scale bar in (a) applies to (b) also.

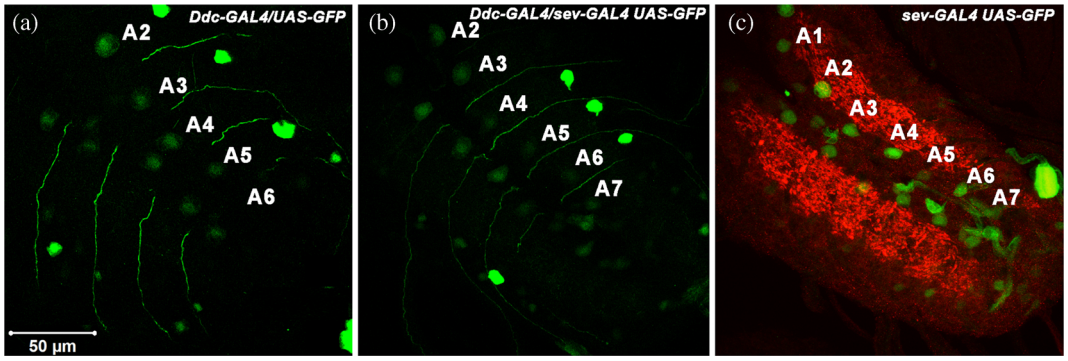

Figure 4. The dorsomedial segmental neurons in ventral ganglia expressing GFP under *sev-GAL4* driver are dopaminergic. (a) *Ddc-GAL4* driven expression of *UAS-GFP* (green) in late larval ventral ganglia; (b) *Ddc-GAL4* and *sev-GAL4* codriven expression of *UAS-GFP* (green) in late larval ventral ganglia; no additional neurons that express GFP (green) are visible; (c) immunostaining for cholinergic neurons (red) of *sev-GAL4>UAS-GFP* expressing (green) late larval ventral ganglia. All the images are confocal projections of several optical sections of the dorsal part of the ventral ganglia. The neuromeric locations (A1–A7) of the dorsomedial paired GFP-positive neurons are marked while the genotype of the respective panel is noted on top-right corner. The scale bar in (a) applies to (a–c).

sev-GAL4 and *Ddc-GAL4* to drive *UAS-GFP* reporter resulted in GFP expression pattern similar to that seen in presence of either only the *Ddc-GAL4* or only the *sev-GAL4* driver (figure 4, a&b). The *sev-GAL4>UAS-GFP* expressing dorsomedial pairs of neurons in ventral ganglia did not show any immunostaining for cholinergic neurons (figure 4c). Thus, these results indicate that the *sev-GAL4* expressing dorsomedial segmental neurons in larval and pupal ventral ganglia are dopaminergic.

Expression domain of sev-GAL4 driver in eye discs and CNS is coincident with that of the endogenous Sev protein

Since the *sev-GAL4* driver transgene includes a single copy of the *sev* enhancer–promoter region to guide expression of GAL4 (Bailey 1999), it is commonly believed that the expression domain of *sev-GAL4* transgene mimics that of the *sev* gene itself. Since our studies revealed expression of GAL4 in specific CNS neurons during larval and pupal stages, we checked if the Sev protein is also present in these neurons through immunostaining of eye discs and the CNS of *sev-GAL4>UAS-GFP* third instar larvae with Sev antibody (figure 5). As expected (Tomlinson *et al.* 1987), Sev protein was present at apical surface of the R3, R4, R7 and to a less extent of the R1 and R6 photoreceptor cells of each ommatidial unit so that optical sections of eye discs along the apical plane showed rows of well-arranged circular rings of Sev protein posterior to the morphogenetic furrow (figure 5a). Since the GFP expression in the present *UAS-GFP* reporter is nuclear, GFP fluorescence in the same photoreceptors was seen in more medial optical sections (figure 5b) which did not show Sev signal. To confirm that the nuclear GFP signal indeed originated from the photoreceptor cells with Sev signal at their apical plane, we examined apico-basal plane

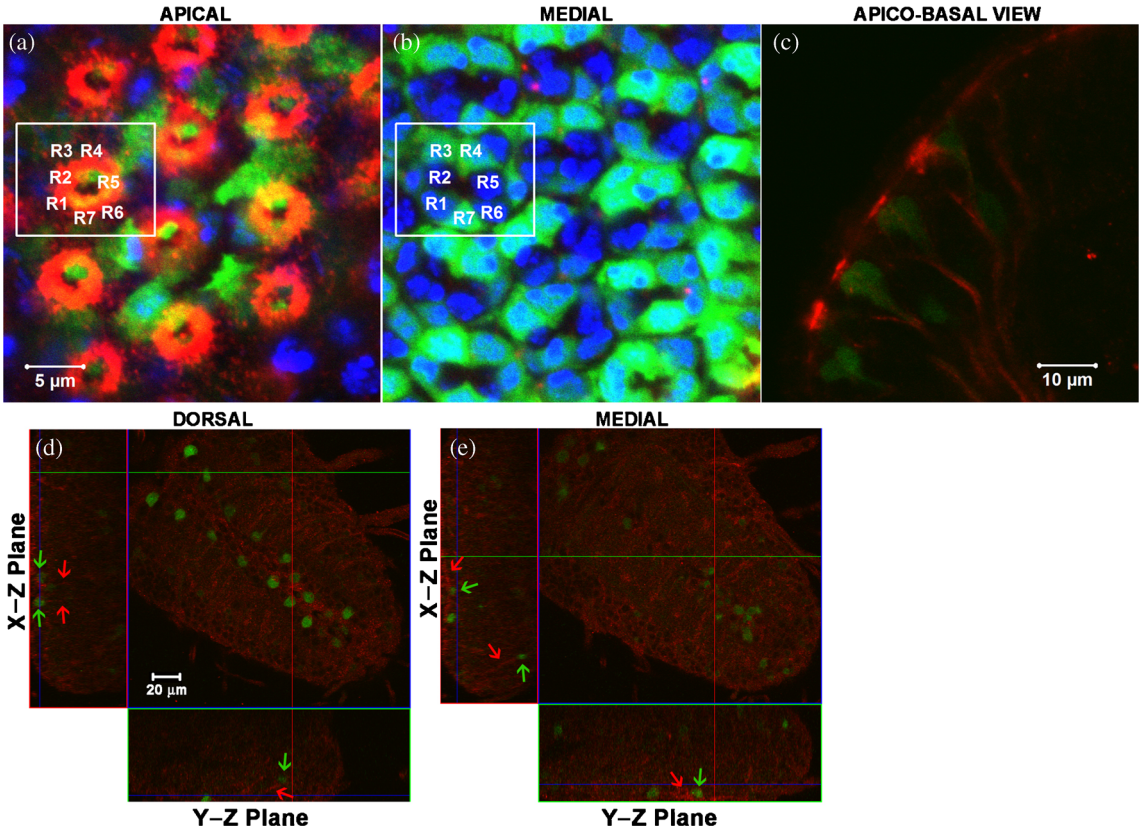

Figure 5. Sevenless protein is present in eye disc and CNS cells that show *sev-GAL4* driven *UAS-GFP* expression. (a–c) Optical sections of *sev-GAL4* driven *UAS-GFP* expressing (green) late third instar larval eye discs immunostained for the Sev protein (red); (a) and (b) are apical and medial optical sections, respectively, of the same eye disc while (c) is an optical section in apico-basal plane of rhabdomeres. The different photoreceptor cells (R1–R7) are marked in one of the ommatidial units in (a) and (b). (d–e) Confocal optical and orthogonal (X–Z (left) and Y–Z (lower) planes sections of dorsal (d) and medial (e) regions of a *sev-GAL4* driven *UAS-GFP* expressing (green) third instar larval ventral ganglia immunostained for Sev protein (red); the red and green lines in the optical section image indicate the X and Y axes, respectively, for which the Z-axis stacks are shown in the orthogonal X–Z and Y–Z plane images; blue lines in the orthogonal images indicate the plane of the optical section. Note the stronger red Sev signal (red arrows) adjacent to green fluorescent (green arrows) neurons and in fibrous tracks, several of which emanate from the green fluorescing neurons. Scale bar in (a) applies to (a and b) while that in (d) applies to (d–e).

optical sections of eye discs (figure 5c) which clearly showed that the Sev and GFP fluorescence signals were emanating from the same photoreceptor cells.

Also in the CNS, the *sev-GAL4>UAS-GFP* positive neurons showed stronger staining for Sev protein (figure 5, d&e). Fibrous tracts with stronger Sev staining appeared to be emanating from these neurons, especially in sections from the medial parts of ventral ganglia (figure 5, d&e). As noted above for eye discs, the nuclear GFP and cytoplasmic Sev protein did not appear to be typically colocalized. However, orthogonal views (X–Z or Y–Z planes) of the stacks of confocal optical sections, clearly revealed their common presence in specific neurons and their axonal projections (figure 5, d&e).

Besides the eye discs, GMR-GAL4 drives UAS-GFP expression in several other tissues, including the sev-GAL4 expressing CNS neurons

As in the case of *sev-GAL4*, we examined the expression domains of the two *GMR-GAL4* drivers through the *UAS-GFP* reporter. It was seen that besides the earlier noted expression in eye discs and optic lobes (figure 1), both the *GMR-GAL4* drivers led to GFP expression in CNS in a pattern similar to that of *sev-GAL4* driver (compare figures 2 and 6; see table 1). To know if the *sev-GAL4*-driven GFP expressing neuronal cells in CNS are same as those seen with the *GMR-GAL4* driver, we generated w^{1118}; *sev-GAL4 UAS-GFP/ GMR-GAL4*^{2} progeny through appropriate genetic crosses so that the *UAS-GFP* transgene was simultaneously driven by both the GAL4 drivers. It was seen that the pattern of GFP expression in the ventral ganglia in these larvae and pupae remained similar (not shown) to that when the *UAS-GFP* transgene was driven by any one of the drivers. This indicates that *sev-GAL4* and *GMR-GAL4* drivers activate *UAS-GFP* in a common set of neuronal cells in ventral ganglia.

In addition, two *GMR-GAL4* drivers also expressed in a common or in transgene insertion-specific manner, in several other embryonic, larval and pupal tissues (table 1; figure 7). Both the *GMR-GAL4* drivers activated GFP expression in embryonic eye primordia (figure 7, a&b), the eye discs in

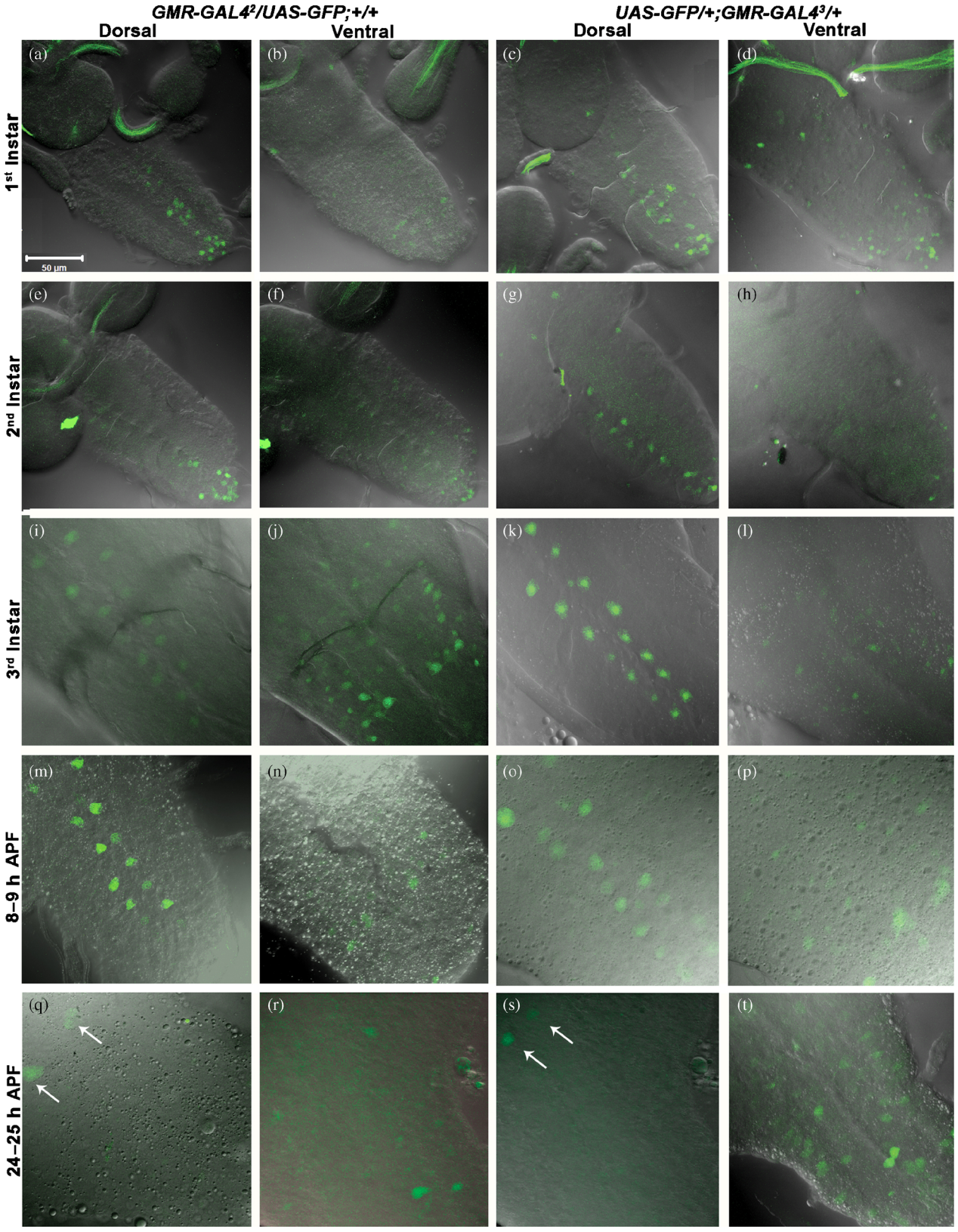

Figure 6. *GMR-GAL4* drivers activate *UAS-GFP* in larval and pupal ventral ganglia in a pattern similar to that seen in *sev-GAL4* driver. Confocal projections showing GFP expression (green, against DIC background) driven by *GMR-GAL4*^{2} (a, b, e, f, i, j, m, n, q and r) or by *GMR-GAL4*^{3} (c, d, g, h, k, l, o, p, s and t) in dorsal or ventral sides (noted above each column) of ventral ganglia from different larval and pupal stages (noted on left of each row). Arrows in (q) and (s) point to the persisting dorsomedial pair of ganglia in T3 neuromere. The scale bar in (a) applies to all the images.

1st and 2nd instar larvae (figure 7, c, f, i&j), Bolwig's organ with its nerve (embryonic and later stages) and in neuronal precursors of ocelli present in between the two optic lobes (figure 7, k, l, m & n). The *GMR-GAL4* driven *UAS-GFP* expression in Bolwig's organ and ocelli is expected since the *GMR-GAL4* (Freeman 1996) construct contains a pentamer of truncated glass-binding sites derived from the *Drosophila* Rh1 promoter (Hay *et al.* 1994) and glass protein is known to be expressed in these tissues (Moses *et al.* 1989; Moses and Rubin 1991). A few other unidentified embryonic cells also

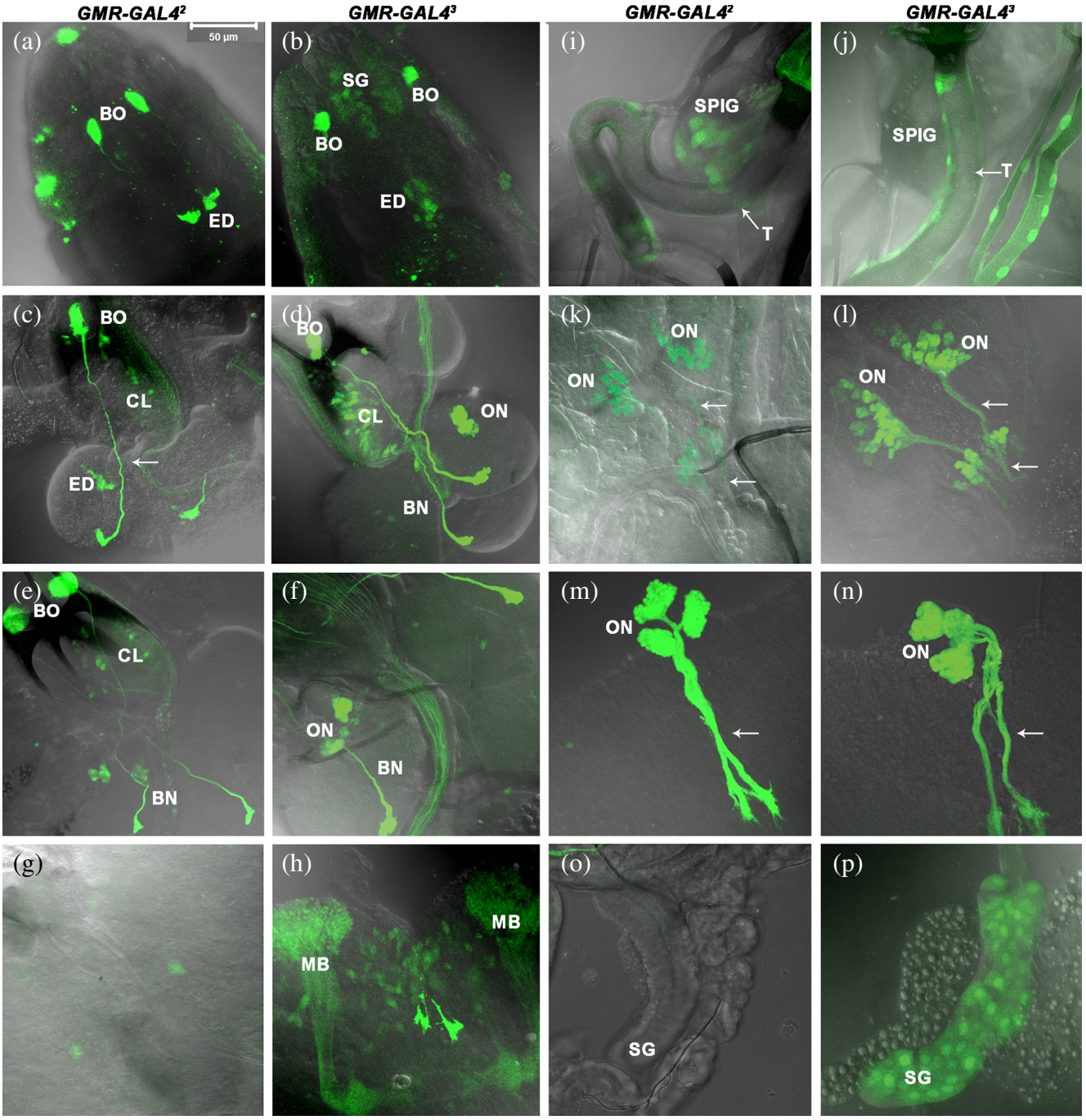

Figure 7. The two *GMR-GAL4* drivers express in several other embryonic and larval tissues in common or in insertion line-specific manner. Confocal projection images show *UAS-GFP* reporter (green) expression driven by *GMR-GAL4*^{2} (a, c, e, g, i, k, m and o) or *GMR-GAL4*^{3} (b, d, f, h, j, l, n and p) driver (noted above each column) in embryo (a, b), 1st instar (c, d), 2nd instar (e, f), third instar (i, j) larvae, 8–9 h old (k, l) or 24–25 h old (m, n) pupae. (g and h) Confocal projection images of brain ganglia of third instar larvae while (o) and (p) are of 2nd instar larval salivary glands. BO, Bolwig's organ; BN, Bolwig's nerve; CL, clypeolabral organ; ED, eye primorida in embryo or eye disc in larvae; ON, ocelli neurons and their projection axons (arrow); SG, salivary glands; SPIG, spiracular glands at the base of anterior spiracles; T, tracheae. Scale bar in (a) applies to panels (a–p).

showed varying degree of *GMR-GAL4>UAS*-GFP expression (figure 7, a&b).

Interestingly, we noted certain differences between the two *GMR-GAL4* insertion lines in their activation of the *UAS-GFP* transgene. While the *GMR-GAL4*^{2} line showed strong GFP expression in third instar larval spiracular glands (figure 7i), the *GMR-GAL4*^{3} (figure 7j) did not activate *UAS-GFP* in this tissue. On the other hand, the GFP expression in larval tracheae was much more extensive and stronger with *GMR-GAL4*^{3} than with *GMR-GAL4*^{2} driver (figure 7, i&j). Another significant difference between the two *GMR-GAL4* drivers was in the expression of the brain lobes (figure 7, g&h). While in case of *GMR-GAL4*^{2}, only one single pair of neuron in middorsal part showed presence of GFP (figure 7g), large number of neuronal cells and the mushroom body were also distinctly GFP-positive in the *GMR-GAL4*^{3} (figure 7h). Further, GFP expression was seen in embryonic (figure 7b) and early larval stage salivary glands (figure 7p) but not in third instar larvae (not shown) in the case of *GMR-GAL4*^{3}; the *GMR-GAL4*^{2} line did not show GFP expression in embryonic or larval salivary glands at any stage (figure 7o).

Discussion

The *sev-GAL4* and *GMR-GAL4* drivers are very extensively used by fly researchers to target expression of the *UAS*-responder transgene to the differentiating eyes. However, our observations clearly show that each of these drivers leads to expression of the reporter gene in several additional tissues, including regions of the CNS. Our study further reveals that the expression domains of the endogenous *sev* gene and the *sev-GAL4* transgene are comparable in eye discs as well as the CNS since the endogenous Sevenless protein was present in all cells that expressed *sev-GAL4* driven *UAS-GFP* transgene. It remains to be seen if the endogenous *glass* gene also expresses in cells expressing GFP under the *GMR-GAL4* driver. It is interesting that the *sev-GAL4* and *GMR-GAL4* drivers share their expression not only in eye discs but also in a common set of neurons in ventral ganglia. This suggests some shared developmental roles of the two genes in these neuronal cells. However, unlike the *sev-GAL4* driver, the *GMR-GAL4* driver is active in several nonneuronal tissues as well.

Recently, Li *et al.* (2012) also reported that the *GMR-GAL4* driver causes the *lacZ* reporter gene to express in wing and leg discs, trachea, etc., besides the eye discs. They did not report expression of the *GMR-GAL4* driver in the CNS. However, unlike their report, we did not find GFP expression in wing or leg discs with either of the two *GMR-GAL4* drivers used by us. Several possible factors may explain the differences between Li *et al.* (2012) and our results. Firstly, the X-Gal-based lacZ reporter assay used by Li *et al.* (2012) is much less sensitive than the GFP-fluorescence assay. The endogenously present fly β-galactosidase can also generate erroneous signals if not properly controlled (Schnetzer and Tyler 1996). The lacZ assay (Li *et al.* 2012) may have failed to detect the weak GFP fluorescence in the neuronal cells of ventral ganglia observed in our study. Finally, our present results with the two *GMR-GAL4* insertion lines reveal that it is possible that different *GMR-GAL4* driver lines may have subtle differences in their expression domains owing to small differences in the transgene construct and/or differences in their site of insertion. Site of insertion of transgene can indeed have positional effects, especially if boundary elements are not placed around the transgene (Labrador and Corces 2002; Srinivasan and Mishra 2012).

Power of the *GAL4-UAS* binary system of targeted gene expression (Brand and Perrimon 1993) lies in the precision of target gene's ectopic expression. However, in the absence of detailed information about the specific expression domain/s of a given GAL4 driver construct, interpretations about functions of a gene or its interaction/s with other gene/s may become misleading. The present results, besides providing detailed information on the expression domains of the commonly used *sev-GAL4* and *GMR-GAL4* drivers, emphasize the need for a careful confirmation of expression domains of the GAL4 driver being used in a given study, rather than relying only on the empirically claimed expression domains.

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