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GAL4* enhancer trap strains with reporter gene expression during the development of adult brain in *Drosophila melanogaster

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Introduction

Understanding the molecular regulation of adult brain development has remained difficult even in a genetically tractable system like *Drosophila*. An important reason for this is that the same genes are deployed repeatedly several times at different stages to carry out different functions in a developmental programme. The classical mutation screens to discover the genetic players often fail to identify the candidate genes taking part in adult development as, in most cases, these mutations lead to embryonic lethality.

Enhancer trap technique developed in *Drosophila* (O’Kane and Gehring 1987; Wilson *et al.* 1989), circumvents the problem of embryonic lethality and allows us to identify and isolate genes based on their expression pattern rather than a mutant phenotype. P-*GAL4*-UAS system is a second generation enhancer system (Brand and Perrimon 1993) which enables us to achieve targeted expression of a gene of interest in specific cell types during development. With an intention to isolate strains that would be useful in understanding the development of the adult brain, strains from a previous P-*GAL4* screen (Shyamala and Chopra 1999) were analysed for developmentally regulated expression of the reporter gene activity in brain. Here, we present the expression pattern of two strains SG26.1 and SG1.1 in central nervous system (CNS), at different time points during the pupal stage—a critical transient stage that transforms the larva into an adult. SG1.1 shows an exclusive cyclical pattern of reporter expression during the pupal stages, whereas SG26.1 has a distinct temporally regulated reporter expression in the optic lobe precursors during pupal development.

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Materials and methods

Fly stocks

Transgenic *Drosophila melanogaster* lines containing random insertion of P-*GAL4* showing adult brain specific *GAL4* expression (Shyamala and Chopra 1999) and transgenic line with UAS nuclear *lacZ* on second chromosome (Brand and Perrimon 1993) were used for this study.

Histochemical detection of activator gene expression

Virgins were collected from w^- ; UAS nuc *lacZ* reporter stock and were crossed to each of the P-*GAL4* transgenic strains. The F₁ larvae and pupae were cultured at 22°C. The formation of white pupa was taken as zero hour and pupae were staged accordingly as number of hours after puparium formation (APF). The pupae were staged inside the culture bottle without disturbing them at different time points. The pupal case was removed and the brain was dissected out from the pupa in PBS. The dissected brains were immediately fixed in freshly prepared 4% paraformaldehyde in phosphate buffered saline pH 7.2 (PBS) for 30 min. The fixed brains were then washed in PBS and stained in X-gal staining solution (0.060 mL 5% X-gal, 0.020 mL 100 mM potassium ferricyanide, 0.020 mL 100 mM potassium ferrocyanide, 0.050 mL 1 M sodium phosphate buffer pH 8.0, 0.850 mL 1× PBS) by incubating them at 37°C. The stained brains were mounted in 70% glycerol, observed and imaged with Olympus BX60 microscope (Olympus Corporation, Tokyo, Japan).

Results and discussion

Pupal stage in *Drosophila* marks a dynamic phase of development wherein a dramatic transformation of the *Drosophila* larva into the adult fly takes place through metamorphosis. In this second phase of morphogenesis, unlike most other larval tissues which are subject to histolysis, the CNS undergoes relatively little cellular death but a major reorganiza-

tion in the existing larval-specific and adult-specific neurons. There is a class of functional larval neurons which survive, withdraw the existing axonal branching and change their arborization to suit the larval-to-adult functional change over (Truman 1990; Lin *et al.* 2009; Singh *et al.* 2010). The larval CNS also contains large number of neuroblasts which are probably specified at the embryonic stage but become mitotically quiescent in the early larva. These cells re-enter the proliferative phase at 12–36 h of larval period and generate clusters of immature postmitotic neurons by late third instar. They do not have any obvious function in larva and are referred to as adult-specific neurons (Truman 1990). These arrested adult-specific neurons begin elaboration of processes during pupariation. Yet another major cellular process observed during pupariation is the removal of a class of larval neurons which are not going to be used in making the adult brain. This occurs through programmed cell death (Truman 1990; Kumara *et al.* 2009). Genetic networks controlling these crucial events during the construction of different neuropiles and functional compartments of the adult brain are poorly understood. Screen for new marker strains specific to pupal stages of development is an important requirement towards getting insights into the problem. Here, we present two new P-*GAL4* enhancer-trap strains which show region specific reporter gene expression in the brain during metamorphosis.

SG26.1 and SG1.1 are two strains with random P-*GAL4* insertions. *GAL4* is a yeast transcription factor that can activate genes downstream of *GAL4* specific upstream activation sequence (UAS). To visualize the expression pattern of the *GAL4*, we have crossed these strains with a transgenic strain carrying *Lac-Z* gene coding for *E. coli* β -galactosidase, downstream of the *GAL4* specific UAS from yeast (Brand and Perrimon 1993). The F₁ individuals will have β -galactosidase expressed in those cells wherever *GAL4* is expressed by the local genomic enhancer.

SG26.1 – an optic lobe specific strain

This strain has a viable P-*GAL4* insertion on the third chromosome. The adult in this strain has reporter gene expression in the pedicel of the antenna, antennal nerve, mechanosensory and motor centres of the brain (Shyamala and Chopra 1999). The 0-h pupal brain shows very strong reporter expression in the larval optic focus which extends laterally as a stripe towards the outer optic anlagen (figure 1a). In addition to this predominant expression, there are small clusters of cells with reporter activity situated in the central brain (cb) and in the suboesophageal ganglion (sog). At 6 h APF stage, there is an expansion of the optic expression domain. The expression which was strong in the inner optic focus is enhanced further and expanded into the lateral region. These cells probably represent lineage-specific, differentiating secondary or the adult-specific optic neuroblasts. The ventral ganglion has number of scattered cells which appear like the primary neuroblasts that are subject to remodelling after their

larval life (figure 1b). By 13 h of pupal development we see a striking enlargement of the expression domain particularly, the one towards the outer optic anlagen (figure 1c). The small clusters in the central brain region and a fewer number of primary neuroblasts continue to show reporter activity. At 24 h stage pupal brain, the expression is more or less restricted to the developing optic medulla and the lobula plate (figure 1d). The expression is enhanced in the lobula region during 63 h stage (figure 1e), which gradually comes down towards the completion of pupal stage (113 h).

SG1.1 – a strain with cyclical expression pattern of the reporter gene

This is a strain which has P-*GAL4* insertion on the third chromosome. The insertion is homozygous lethal. We see interesting oscillations in the expression levels of the reporter gene throughout the pupal development in this strain. The expression at 0-h pupa is strong in the mushroom body (mb), cb, sog and in the interhemispheric junction (ij) (figure 2a). At 20 h APF, there is a decline in the level of expression in the cb and sog, where as it remains very strong in the olfactory/mushroom body region (figure 2b). At 24 h APF, there is almost complete withdrawal of the reporter expression and the brain shows very faint staining in the expected domains (figure 2c). A second wave of increased expression appears at 30-h pupal stage, with strong expression in all the previously marked domains (figure 2d). At 41 h there is a striking decline in the reporter expression (figure 2e). The gene activity reappears by 43 h stage and reaches the peak at 48 h (figure 2, f&g). From 69–94 h stage we see a gradual reduction in the expression of the reporter gene (figure 2, h,i&j). The next bout of gene activity is seen in the brain of late pupa (101-h APF). Here strong expression is seen in most of the cortex region with a more pronounced activity in the mushroom body area (figure 2k). So far, there are no such cyclical cellular processes which have been reported to be occurring during the pupal development in brain. The cyclical oscillations of the reporter gene activity which in turn may be a representation of the similar oscillating activity of the native gene at the site of insertion is very intriguing.

Studies have been carried out by various workers (Hartenstein *et al.* 2008; Lichtneckert and Reichert 2008; Urbach and Technau 2008; Rodrigues and Hummel 2008) towards understanding the development of adult brain in *D. melanogaster*. Attempts have been made and several genes have been identified as involved in different aspects of *Drosophila* adult brain development. Genes like *mushroom body defective* (*mud*), *anachronism* (*ana*), *terribly reduced optic lobe* (*trol*), *baboon* (*babo*), *eyeless* (*ey*), *mini-brain* (*mnb*), *wingless* (*wg*), *DE-cad*, *Grainy head* (*grh*), have been shown to be involved in larval brain neuroblast proliferation (Prokop and Technau 1994; Brummell *et al.* 1999; Kurusu *et al.* 2000; Tejdor *et al.* 1995; Hartenstein *et al.* 2008). Homeobox containing genes such as

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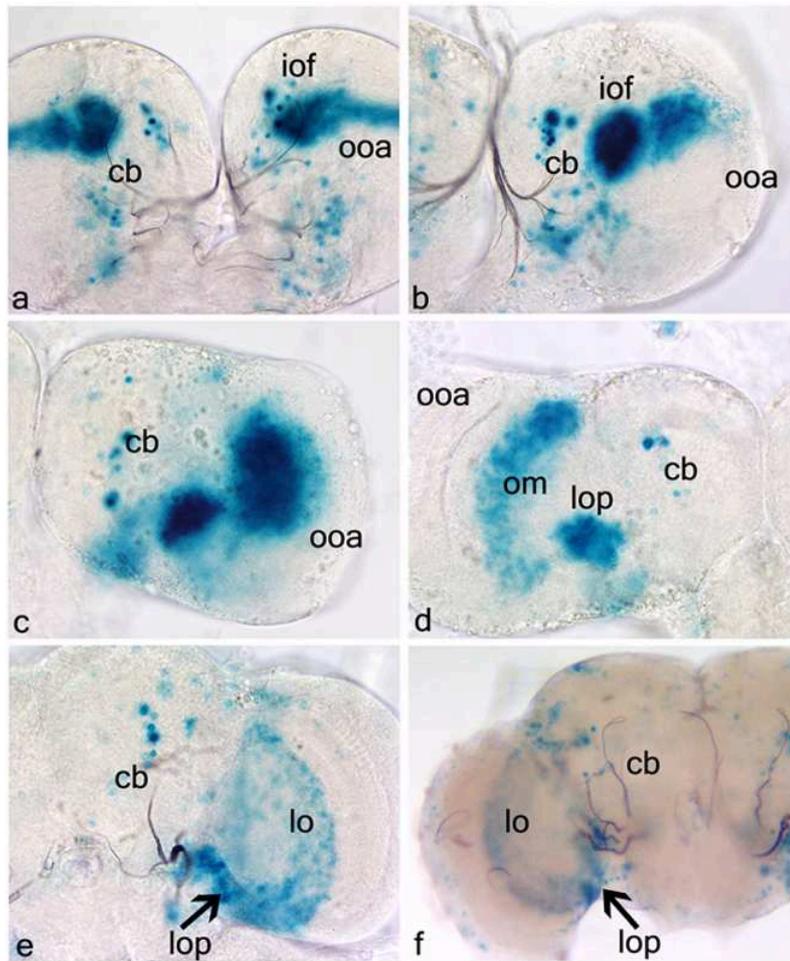


Figure 1. GAL4 expression pattern in the pupal brain of SG26.1 *GAL4* enhancer trap strain. SG26.1 was crossed to, UAS-Nuc *LacZ* strain and the F₁ pupal brains at different stages were stained for β -galactosidase activity. (a) 0-h pupal brain in dorsal view, expression of reporter gene can be seen in inner optic focus (iof). The expression extends as a stripe towards the outer optic anlagen (ooa). (b) Dorsal view of brain at 6-h APF, stronger reporter expression is seen in the inner optic focus (iof) which extends laterally. Few cells in central brain (cb) and sog have reporter gene expression. (c) Dorsal view of pupal brain at 13-h APF, reporter expression is enlarged into broad domain particularly at outer optic anlagen (ooa). (d) Dorsal view of 24-h APF stage brain showing reporter expression in optic medulla (om), lobula (lo) and in lobula plate (lop). (e) Frontal view of left brain lobe at 63-h APF with reporter gene expression in lobula (lo) and lop. (f) 96-h APF staged pupal brain in frontal view, with further reduced reporter gene expression.

Orthodenticle and *empty spiracle* are implicated in anterior/posterior specification of the developing brain (Lichtneckert and Reichert 2008). *Atonal*, *robo* and *notch* expressions are needed for establishing specific cell fates and connectivity in the olfactory lobe (Jhaveri *et al.* 2004; Das *et al.* 2010). Relatively more number of genes taking part in optic lobe development have been uncovered (Fischbach and Hiesinger 2008; Malartre *et al.* 2010; Chang *et al.* 2010). But the picture that we have of the complex process of making an adult CNS is far from complete, and hence there is a continued need for identification of marker strains and genes cor-

responding to the development of adult brain in *Drosophila*. Pupal expression patterns of reporter gene presented here are important as they give us a lead to identify the native genes which are present in the vicinity of the respective P-insertion and are expressed in the same pattern as that of the reporter. As the strains described here are targeted expression systems (Brand and Perrimon 1993), they will also be useful for carrying out targeted misexpression / ectopic expression studies during the pupal stage for any gene of interest in brain—thus can be useful tools in our efforts to understand the adult brain development in *Drosophila*.

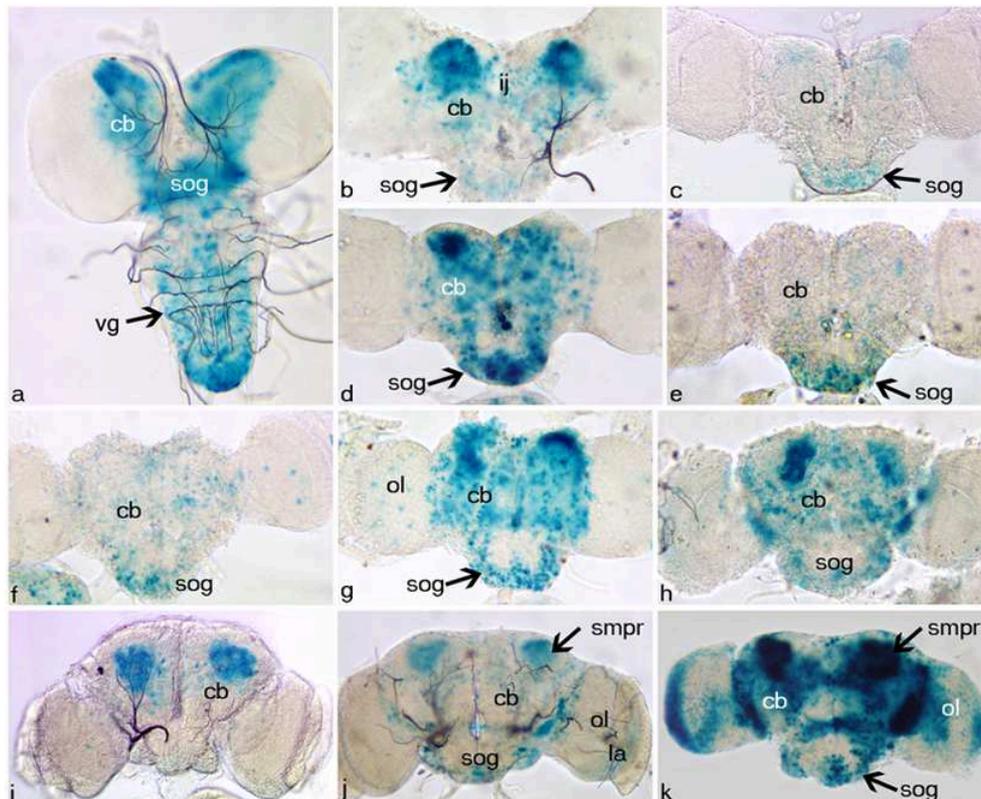


Figure 2. Cyclical pattern of GAL4 expression in the pupal brain of P-GAL4 enhancer trap strain. SG1.1 was crossed to, UAS-Nuc LacZ strain and the F₁ pupal brains at different stages were stained for β-galactosidase activity. (a) 0-h APF staged pupal brain in dorsal view; reporter gene expression is seen in central brain (cb), suboesophageal ganglion (sog), and in entire ventral ganglion (vg), with pronounced expression in the posterior region of the vg. (b) 20-h APF with reporter expression seen in cb, interhemispheric junction (ij) and in sog. The expression has decreased compared to the 0-h stage. (c) Dorsal view of brain at 24-h APF stage with weak reporter expression in anterior central brain region and in sog. The expression level is very weak compared to the 20-h APF stage. (d) Dorsal view of brain at 30 hrs APF, second wave of reporter expression is seen which is very strong in cb and sog. (e) 41-h APF staged pupal brain in dorsal view. Expression is almost undetectable in cb and weak in the sog. (f) 43-h APF staged pupal brain in dorsal view with decreased levels of reporter expression in cb and sog. (g) Dorsal view of brain at 48.5-h APF stage exhibiting the next cycle of strong expression of reporter gene in cb, and sog. Few cells in optic lobe (ol) also show reporter expression. (h) 69-h APF staged brain in dorsal view with reporter expression in cb and sog. The expression level is weaker compared to the 48.5 h stage. (i) 73-h APF staged brain in posterior view; reporter expression is present only in anterior cb which is weaker compared to the 69-h APF stage. (j) 94-h APF staged brain in frontal view; weak reporter expression is seen in superior medial protocerebrum (smpr), cortical regions of cb, sog and lamina (la). (k) 101-h APF staged brain in frontal view with very strong reporter gene expression in smpr, cortical regions of cb, sog and in ol.

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