Reporter gene expression in pupal stages of a gustatory specific *P-GAL4* enhancer trap strain of *Drosophila melanogaster*.

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Enhancer trap system developed in *Drosophila* (O'Kane and Gehring, 1987) serves as a tool to identify the genes primarily based on their expression pattern, thus allows us to circumvent the problem of embryonic lethality that is usually caused by mutations, and enables us to look into their functions at later stages of development. A *P-GAL4* screen (Brand and Perrimon, 1993) was done for the enhancer traps with reporter gene expression in the adult brain of *Drosophila melanogaster* (Shyamala and Chopra, 1999). Here we report the pupal expression pattern of the reporter gene in an adult Maxillary palp and taste nerve specific strain.

The F1 embryos of the cross between *P-GAL4* strain SG17.1 and the UAS *Lac-Z* strain were collected and cultured at 22°C. The formation of white pupa was taken as zero hour, and pupae were staged accordingly as the number of hours After Puparium Formation (APF). The staged pupae were dissected out, and the brain along with ventral ganglion was stained for β-galactosidase activity using the standard protocol (VijayRaghavan *et al*., 1986).

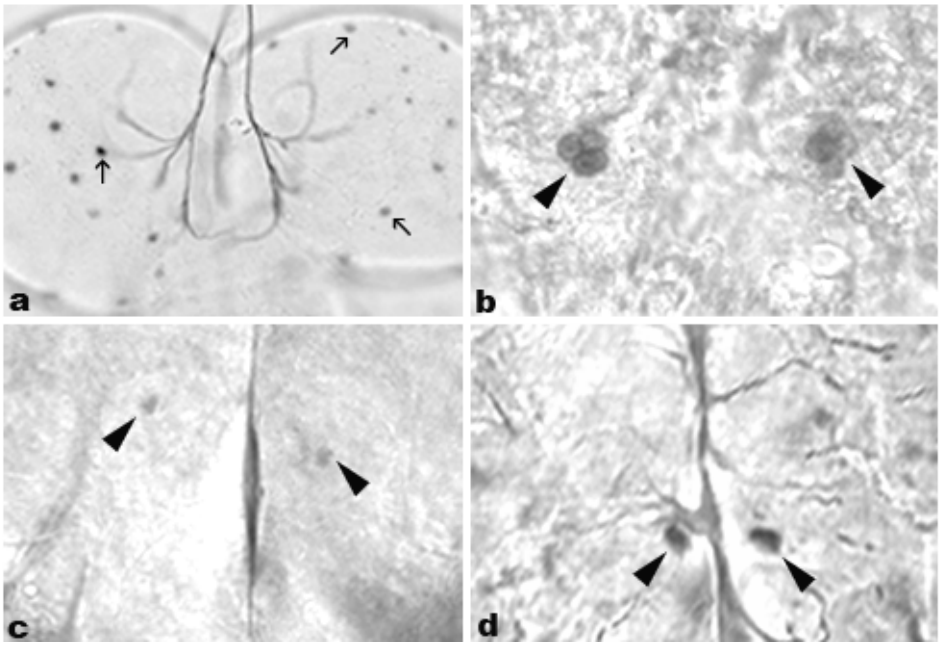

Figure 1. Brain of *P-GAL4* insertion strain SG 17.1 during pupal stages. Arrows and arrowheads indicate cells with reporter gene expression. a) 0 hr APF in dorsal view (DV), cells are seen scattered in entire brain. b) 43 hr APF stage, suboesophageal ganglion region shown in DV, a cluster of four cells can be seen on the lateral side. c) 96 hr APF stage, frontal view (FV) of central complex region. d) pharate brain in FV cells with reporter gene expression are seen in the central complex region.

SG17.1 is a homozygous viable strain with *P-GAL4* insertion in the X-Chromosome. At 0 hr pupal brain (Figure 1a), reporter gene expression is seen in cells which are more or less uniformly scattered in the entire brain and Ventral ganglion. The number of cells with expression is slightly higher on the dorsal side of the ventral ganglion. During 6, 14, and 24 hr (images not shown), a similar pattern as that of the zero hr stage is seen with a reduction in the intensity and the number of cells expressing the reporter gene. At 43 hr stage (Figure 1b) expression gets confined predominantly to a pair of cell clusters at the suboesophageal ganglion (SOG) (Truman, 1990), whereas it is withdrawn from rest of the brain regions. This expression in the SOG cell clusters at 43 hr seems to be transient, as it is not seen in the later stages. Interestingly, in the 65 hr pupal brain, the global scattered expression pattern is reiterated (image not shown). At later stages, transient expression in isolated cells in the antennal lobe, superior medial Protocerebrum (Ref: Flybrain) and the SOG is seen. Figures are shown for 96hr (Figure 1c) and pharates (Figure 1d).

Earlier studies on the adults of this particular strain have shown reporter gene expression in the components of the gustatory system, including maxillary palp and taste nerve, third segment of the antenna, olfactory region, and Calyx in the brain (Shyamala and Chopra, 1999). The present finding as to its expression during pupal stages implies a developmental role to the native gene at the site of *P-GAL4* insertion.

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References: Brand, A.H., and P. Norbert 1993, Development 118: 401-415; O'Kane, C.J., and W.J. Gehring 1987, Proc. Natl. Acad. Sci. USA. 84: 9123-9127; Shyamala, B.V., and A. Chopra 1999, J. Genet. 78: 87-97; Truman, J.W., 1990, J. Neurobiol. 21: 1072-1084; Vijayraghavan, K., M.A. Crosby, P.H. Mathers, and E.M. Meyerowitz 1986, EMBO J. 5: 3321-3326; Flybrain http://flybrain.neurobio.arizona.edu.

The *mus309* mutations, defective in DNA double-strand break repair, mobilize the *gypsy* element in *Drosophila melanogaster*.

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In *Drosophila melanogaster* the mutagen-sensitive *mus309* locus on the third chromosome right arm (86F4) encodes, in a manner similar to its orthologues in other organisms, the mammalian *BLM* locus included, a RecQ helicase (Boyd *et al.*, 1981, 1987; Ellis *et al*., 1995; Karow *et al*., 1997; Moghaghegh *et al*., 2001; Wu *et al*., 2001) and, accordingly, is involved in DNA double-strand break repair (reviewed in Brabant *et al*., 2000; Heyer *et al*., 2003; Heyer, 2004).

The *mus 309* mutation is also known to be defective in double-strand DNA break repair after P element excision (Beall and Rio, 1996; McVey *et al*., 2004).

The ct^6 mutation of *Drosophila melanogaster* (*ct*, cut 1 – 20.0) for its part is caused by an insertion of the mobile *gypsy* element (Lindsley and Zimm, 1992).

My intention was to construct two *w* ct^6*; mus309/Tb* stocks (*w*, white 1 – 1.5; *Tb*, Tubby 3 – 90.6) carrying either the $mus309^{D2}$ or the $mus309^{D3}$ allele, both of which are sterile in females and semi-sterile in males (Boyd *et al*., 1981; Kusano *et al*., 2001).