

# Conditional UAS-targeted repression in *Drosophila*

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## ABSTRACT

The Gal4–UAS enhancer trap system is useful for driving gene expression in various tissues. A new tool that extends Gal4 technology is described here. A fusion protein containing the Gal4 binding domain and the repression domain of the isolator suppressor of hairy wing was placed under the control of a heat shock-inducible promoter. The construct mediates the conditional repression of genes located downstream of a UAS sequence. The repressive effects of the chimeric protein on *fasII* gene expression were tested by western-blot analysis and in brain sections of adult *Drosophila*. Owing to the increasing number of Gal4 and UAS transgenic lines, this versatile system will facilitate the study of gene function.

## INTRODUCTION

A vast variety of techniques to study gene function are available for *Drosophila*. In classical genetic screens, genomes can be mutagenized using chemicals, ionizing radiation or transposable elements (1,2). This approach typically produces mutations leading to total or partial loss-of-function (LOF). Constitutive LOF mutations are of limited use for the study of physiological functions in adult flies because they are often associated with developmental lethality or structural anomalies. This limitation can be overcome by the specific repression of genes at the adult stage or in specific tissues. Clonal analysis allows LOF phenotypes to be studied in specific cells, but this approach is generally unsuitable for analysis of complex phenomena such as behavior because the mutant cells are generated randomly and asymmetrically. Recently, RNA interference (RNAi) has been developed as a powerful inducible tool for gene function analysis (3).

The Gal4 enhancer trap strategy offers an alternative, as it allows for gain-of-function (GOF) analysis of proteins that can be expressed under the control of 'targeted' P-elements insertions carrying the UAS sequence (4–7). With this system,

proteins can be overexpressed in different tissues or at different times in the *Drosophila* life cycle (8). Here, we describe an improvement of the Gal4 enhancer trap technology. We have developed a chimeric protein carrying the Gal4 binding domain (GAL4BD) region inserted into the isolator domain of the suppressor of hairy wing protein (SUHW). The construct is under the control of a heat shock promoter, allowing for the conditional expression of the isolator.

## MATERIALS AND METHODS

### *Drosophila* stocks and heat shock treatment

The *Drosophila* stocks were maintained on a 12:12 dark/light cycle on standard cornmeal-yeast agar medium at 25°C. The wild-type strain was *Canton-Special* (CS). Two *fasciclinII* (*fasII*) lines carrying P(UAS) sequences were used. Both *fasII* P(UAS) insertions affect *fasII* expression only slightly (data not shown). The *fasII*<sup>EP1462</sup> line was provided by the Bloomington stock center, and the *fasII*<sup>GS1069</sup> line was obtained from the collection of T. Aigaki (Tokyo Metropolitan University, Tokyo). Both lines were out-crossed with the wild-type strain CS for five generations. For heat shock (HS) experiments, 3–5-day-old flies were placed into tubes pre-heated at 37°C and incubated in a 37°C bath with agitation. For a typical experiment, two 15 min HSs 30 min apart were used.

### Molecular techniques

The following oligonucleotides were used for PCR:

GAL4BDF: 5'-AACATATGAAGCTACTGT-3'  
GAL4BDR: 5'-AACCGCGGCATACAGTC-3'  
SURD5F: 5'-TTGAATTCACCAACATGAGTG-3'  
SURD5R: 5'-TTCATATGGTCTCGGTGACAAC-3'  
SURD3F: 5'-AACCGCCGCTCGTGACGAAGGC-3'  
SURD3R: 5'-AATCTAGAACAAGCTTTCTCTTG-3'.

The Gal4BD was amplified from pAS2 (9) with GAL4BDF and GAL4BDR and cloned into pCR2.1 to create pCRGal4BD. The 5' and 3' regions of the repression domain encoded by the Su(HW) cDNA were amplified from

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pGEM-3Z-Su(HW) using SURD5F–SURD5R and SURD3F–SURD3R, respectively. Both fragments were cloned into pCR2.1 to generate pCRSURD5 and pCRSURD3, respectively. The 5' region was excised as a SpeI–NdeI fragment from pCRSURD5, treated with the DNA polymerase Klenow fragment and cloned into EcoRV-/SacII-digested pCRGal4BD. The resulting plasmid was opened with SacII and KpnI and ligated to the SacII–KpnI fragment of pCRSURD3 containing the 3' region of Su(HW). The open reading frame (ORF) encoding the chimeric protein was excised from the resulting plasmid with EcoRI and SmaI and cloned into the vector pCASPER-hs digested with EcoRI and HpaI. All PCR fragments and cloning junctions were confirmed by sequence analysis.

### Western-blot analysis

Total proteins were isolated from frozen animals in homogenization buffer (25 mM Tris–Phosphate, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane tetraacetic acid, 10% glycerol and 1% Triton X-100). An aliquot of 50 µg of protein extracts was loaded per lane, and western blots were performed as described previously (10). After protein transfer, membranes were stained with Ponceau red and photographed for protein normalization (GeneGnome; Syngene USA, Frederick, MD). Monoclonal antibodies against the Gal4 (1:500; BD Biosciences, USA) and FasII (1:1000) proteins were used. Western

blots were developed using SuperSignal West Pico (Pierce, Rockford, IL). The chemiluminiscent signal was acquired with a CCD camera (Syngene) and quantified with Gene Tools software (Syngene). The FasII antibody was kindly provided by C. Goodman.

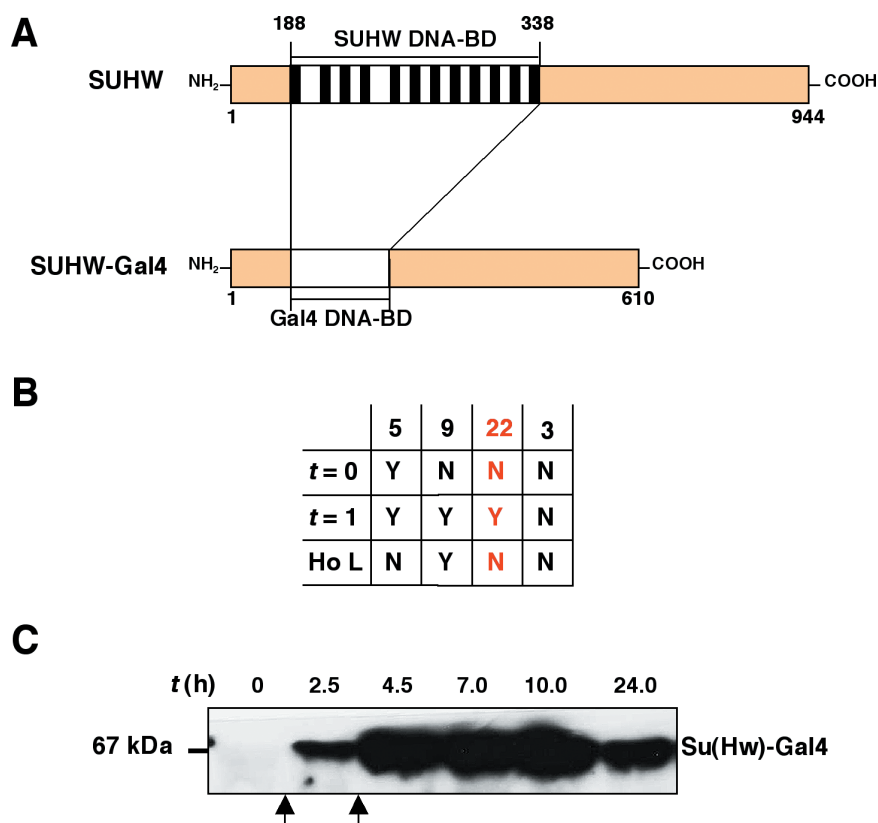
### Immunocytochemistry

Histological collars were prepared by mounting alternatively in the same collar *fasII*<sup>EP1462</sup>/Y; P(hs–SUHW–Gal4)/+ flies that were subjected to HS or left untreated. Adults were embedded in paraffin and sectioned as described (11). Adult 7 µm serial frontal sections were stained with the anti-FasII ID4 monoclonal antibody (12) as described (13), at a dilution of 1:10. Signal was detected using the Vectastain ABC Elite kit (Vector Laboratories, Inc., Burlingame, CA). Expression was monitored under a Leica microscope (Leica Microsystems, Germany).

## RESULTS AND DISCUSSION

### The SUHW–Gal4BD fusion protein is conditionally expressed

To create a system allowing conditional repression in *Drosophila*, we selected the SUHW isolator domain in combination with the Gal4 DNA binding domain. This strategy,



**Figure 1.** The SUHW–Gal4 protein is conditionally expressed. (A) Schematic representation of the SUHW and SUHW–Gal4BD proteins. Numbers correspond to amino acids and the black bars in the SUHW protein represent zinc finger domains. (B) Characterization of transgenic lines. Lines 5, 9, 22 and 3 have independent insertions of P(hs–SUHW–Gal4BD). *t* = 0, repression before HS; *t* = 1, repression 1 h after transgene induction. Y, yes; N, no; and Ho L, homozygous lethal. (C) Expression analysis of line 22. Western blots were performed with Gal4BD monoclonal antibodies. Arrows indicate times at which a 15 min 37°C HS was administered (0 and 2.5 h after the first timepoint).

which allows Gal4–UAS GOF experiments to be carried out in parallel with LOF studies, entails the construction of flies that are homozygous or hemizygous for a UAS-tagged gene and heterozygous for the repressor construct. The *su(Hw)* mutation was isolated as a suppressor of the phenotypes of gypsy insertion alleles. The isolator protein SUHW has the following properties: (i) it binds to a retrotransposon sequence in the gypsy element and silences proximal genes (14,15), (ii) it acts directionally, in that it blocks enhancer–promoter communication only when the SUHW binding site is located between these two regulatory elements (16) and (iii) it binds to DNA using zinc fingers, like the Gal4 transcription factor (17), a feature that increases the likelihood of obtaining a functional chimeric protein. We substituted the zinc fingers of SUHW with those of Gal4–BD and kept the repressor domains entirely intact (Figure 1A). The fusion construct was placed under the control of a HS promoter (pCASPER-hs). *Drosophila* germ line transformation allowed several independent P(hs–SUHW–Gal4BD) insertions to be recovered.

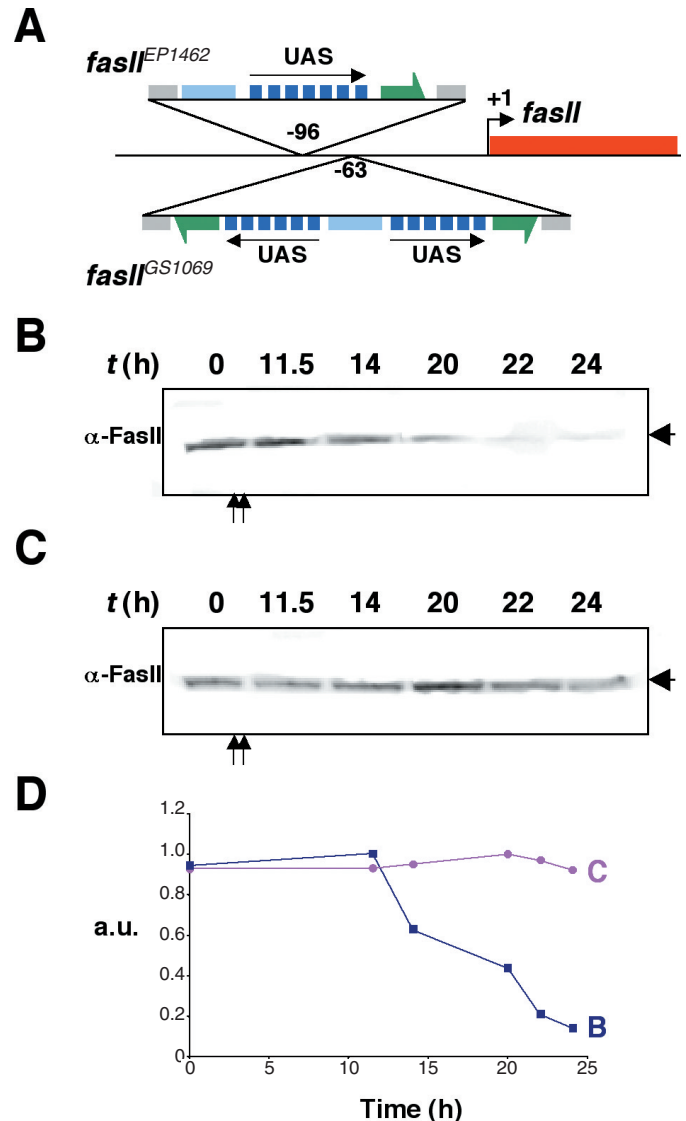
We first studied the expression of the fusion protein in these transformed lines before and after induction by a 15 min 37°C HS. Two lines (9 and 22) out of four tested by western blot using anti-Gal4 monoclonal antibodies proved to be fully conditional (data not shown). Since line 9 was inviable when the construct was homozygous, further studies were carried out with line 22 (Figure 1B). We next optimized the induction conditions. We performed spaced HS to achieve maximal repressor expression. After two 15 min HSs 2.5 h apart, we observed a strong expression of the repressor (Figure 1C). A 30 min interval between the two HSs also led to a strong repressor expression, while shorter intervals between HS treatments were less efficient. Additional or longer HS treatments did not increase the extent of expression (data not shown).

The general state of adult flies appeared normal after HS. We also examined whether the fusion protein affected development. HS performed for 15 min daily during embryonic and early larval life induced no deleterious effect on the normal schedule of development. However, the induction of the repressor at the end of the third larval stage prevented pupariation, resulting in lethality. This effect may be due to titration by the overexpressed protein of partners of the endogenous SUHW protein.

### Adult *fasII* repression

We selected the *fasII* locus to analyze repression mediated by the Su(Hw)RD–Gal4BD fusion protein, because there are two independent lines with P(UAS) insertions in the regulatory region of this gene, *fasII*<sup>EP1462</sup> (6) and *fasII*<sup>GS1069</sup> (7) (Figure 2A), and because *fasII* is on the X chromosome, which allows hemizygous males to be analyzed. Protein extracts from adults were subjected to western blot analysis to determine the kinetics of repression of FasII after HS treatment (Figure 2B and C). A clear decrease was observed at ~24 h, with an average repression at 20–24 h of  $54 \pm 7\%$  ( $n = 5$ ) with *fasII*<sup>EP1462</sup> (Figure 2D). In the absence of the SUHW–Gal4BD repressor, the HS treatment did not affect the level of FasII expression (data not shown). A similar repression was observed with *fasII*<sup>GS1069</sup> (data not shown).

To directly confirm the repressive effect on FasII expression in the brain we analyzed paraffin sections of heads collected

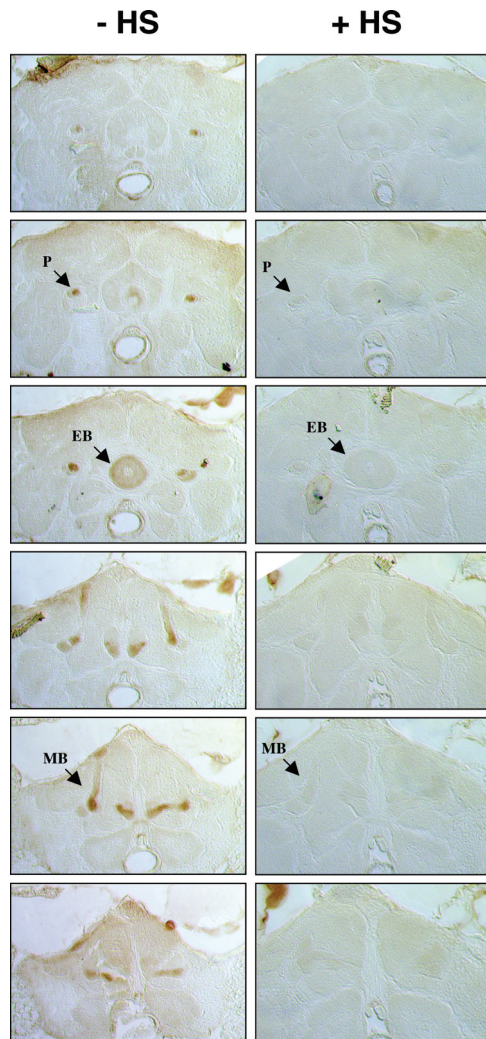


**Figure 2.** Fusion protein induction is correlated with a decrease in the level of FasII protein. (A) Schematic representation of the two *fasII* P(UAS) insertions used in this work. The UAS sequences are in blue and their polarity is indicated by the arrows. A minimal promoter is shown in green. Light blue boxes represent the *white+* marker. The gray boxes represent the P-element ends. Numbers correspond to the relative positions of the P(UAS) insertions in relation to the *fasII* transcriptional start (+1). (B) *fasII*<sup>EP1462</sup>/Y; P(hs–SUHW–Gal4BD)/+ flies were treated with two 15 min HSs at 0 and 30 min (arrows) and whole fly protein extracts were prepared at different timepoints. Immunostaining with anti-FasII antibodies revealed a decreased amount of the FasII protein (arrowhead). Similar kinetics of repression were seen for the other *fasII* P(UAS) line (data not shown). (C) *fasII*<sup>EP1462</sup>/Y; +/+ control flies were treated and immunostained as in (B). (D) Quantification of the data presented in (B and C).

24 h after HS treatment. As shown in Figure 3, the induction of the fusion protein correlated with a strong decrease in FasII immunoreactivity in brain structures where the protein is normally expressed (Figure 3).

This new technique adds inducible LOF to GOF analysis, thereby expanding the utility of the Gal4 enhancer trap system. The ability to use the same P(UAS) insertion to down-regulate or up-regulate the expression of a given gene will help in





**Figure 3.** FasII protein levels in the brain are reduced by transgene induction. Frontal paraffin head sections of *fasII<sup>EP1462</sup>/Y*; P(*hs-SUHW-Gal4BD*)/+ 3 day-old flies without (–) or with (+) two 15 min HSs spaced 30 min apart. FasII expression was detected by immunostaining with the anti-FasII monoclonal antibody. EB, ellipsoid body; MB, mushroom body; and P, peduncle.

correlating the level of a protein with its physiological function.

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