An undergraduate laboratory exercise aimed to demonstrate regulation of eukaryotic gene expression using the GAL4-UAS system in *Drosophila melanogaster*.

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Abstract

The aim of this laboratory exercise is to demonstrate the genetic aspects of gene regulation using transgenic approaches in *Drosophila melanogaster*. During this exercise the students learn about the life cycle and morphology of *Drosophila* and aspects of Mendelian genetics, while testing effects of mis-expression of genes by means of observing phenotypes. The students are directed to set up simple genetic crosses and analyze the effects of mis-expression of genes that cause cell death or are responsible for eye determination. This exercise uses the GAL4-UAS system, a well-established system in flies, to express genes in a tissue or cell-specific manner. This exercise is convenient for an undergraduate lab set up owing to the short generation time of the model organism, ease of manipulation, and amplification for both large and small group set ups. At the end of this exercise the students learn about the mechanisms involved in spatial (domain specific) and temporal regulation of different genes during development. More specifically, they study the role of regulatory sequences (cis-acting elements) and transcription factors (trans acting elements) in defining tissue specificity. These aspects of eukaryotic gene regulation are elucidated by means of reverse genetics that involve mis-expression of genes involved in cell death and cell fate determination.

Introduction

An important aspect of most undergraduate genetics courses is the study of prokaryotic and eukaryotic gene regulation. We devised a laboratory exercise to demonstrate the role of regulatory elements in specific tissues for our Genetics Laboratory course. We designed this lab exercise using the GAL4-UAS system (Brand and Perrimon, 1993) that allows ectopic expression of genes. The exercise involved over-expression (gain-of-function) of specific genes that caused observable phenotype. All cells gain their identity based on the characteristic expression profile of genes. Our knowledge of gene function has traditionally depended on analysis of null phenotypes caused by loss of function of genes. However, sometimes loss of function has no apparent phenotype due to genetic redundancy or it causes lethality. In these scenarios, gain-of-function approach is also an informative tool to study the effects of gene expression in a cell (Phelps and Brand, 1998). Consequently, gain-of-function approaches have also provided important insights into gene function and regulation. Thus, both loss-of-function and gain-of-function methodologies provide important insights into gene regulation and function that generate specific cell fates.

In the lab exercise, we use the bipartite GAL4-UAS system, where one transgenic fly harbors the GAL4 gene under the control of a specific promoter, while the other transgenic fly harbors a target gene fused to GAL4 binding sequences called the Upstream Activator Sequence (UAS) (Figure 1). In the system generated by Brand and Perrimon, the gene of interest is cloned into the polylinker (multiple cloning site, MCS) of the vector p[UAST] downstream of the five optimized GAL4 binding

sites. This construct is microinjected into *Drosophila* embryos to generate transgenic flies that harbor Upstream activating Sites (UAS) and the gene of interest. Both the GAL4 and the UAS transgenic flies are viable as the genes are not constitutively active in individual flies. This helps to maintain lethal mutations and study their function in tissue specific manner. When these transgenic lines are crossed to each other then the Gal4 and the UAS transgenes are both expressed in trans-heterozygous flies of the F1 generation (Figure 1). The GAL4 protein dimerizes and binds to the UAS sites and drives the expression of the downstream gene. If the mutation is not lethal, then both the GAL4 and the UAS sequence can be recombined into one fly.

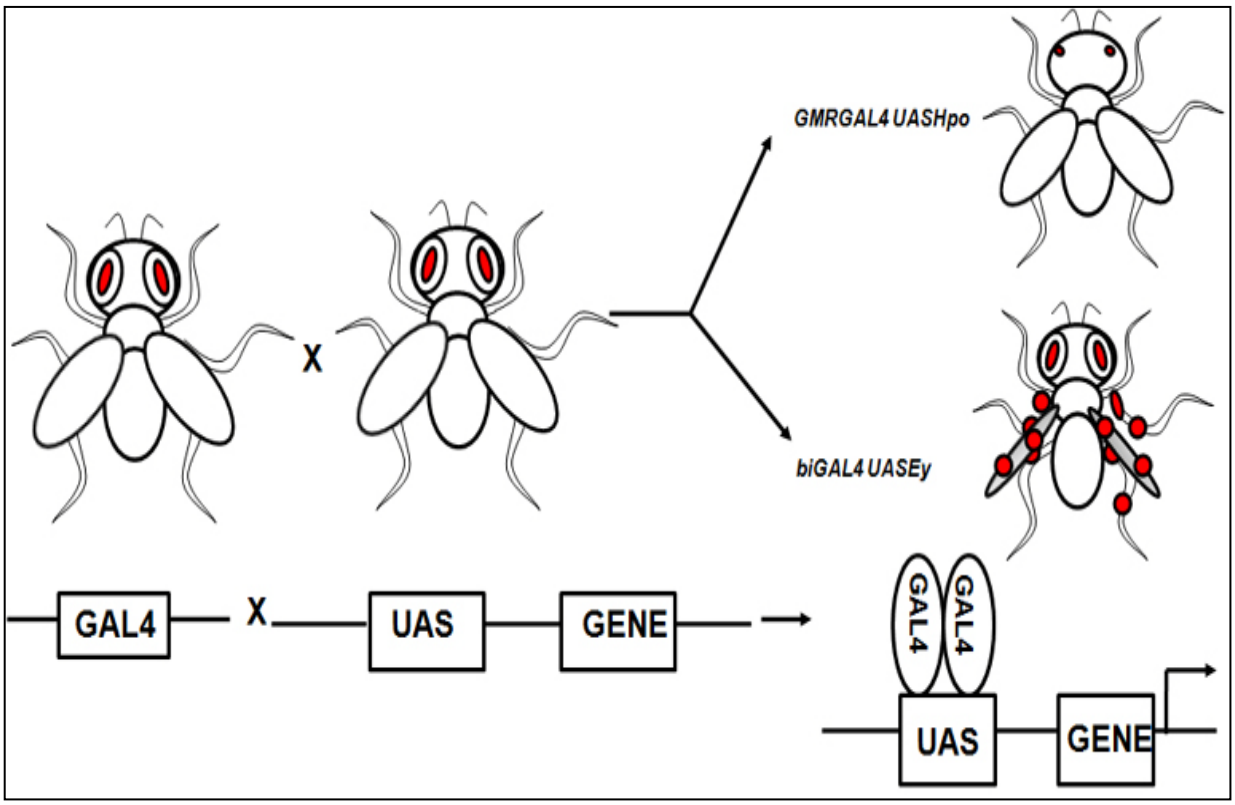

Figure 1. A model of the GAL4-UAS system: The fly harboring the GAL4 gene linked to a tissue-specific promoter is crossed to a fly harboring the gene of interest downstream of the UAS sequence. The progeny for *GMRGAL4 UASHpo* show small necrotic eyes, while the progeny for *biGAL4 UASEy* show ectopic eyes on legs, halteres, and wings as demonstrated in the figure.

Prior to proceeding to the lab session, the students are expected to know the general structure of DNA, chromosomes, and concepts related to transgenes, transcription, and translation in eukaryotes. The students are given brief introduction to the genetics laboratory that covers the theoretical aspects and the experimental design. Two sets of experiments are performed, one with the Hippo transgene (UAS-Hpo) (Udan *et al.*, 2003) and the second with the *eyeless* transgene (UAS-Ey) (Halder *et al*., 1995; Kango-Singh *et al*., 2003). Over-expression of *hippo* is known to cause cell death (Udan *et al*., 2003; Verghese *et al.*, 2012). This experiment utilizes the GMR-Gal4, which directs the expression of UAS-linked transgenes in the region posterior to the morphogenetic furrow where photoreceptor cells differentiate (Figure 2c). The over-expression of UAS-Ey is done using

the bi-GAL4 (Figure 2a), which is expressed in the leg, halteres, and wings and can be used for domain specific expression of transgene (Tare *et al*., 2012). The over-expression of Eyeless leads to development of ectopic eyes on legs, halteres, and wings of the flies (Halder *et al*., 1995; Kango-Singh *et al*., 2003).

Experimental Design

These experiments span a period of roughly three weeks (see Table 1). During the first lab session, the students learn to distinguish between the males and females on the basis of the anatomy of the fly. They are also taught to collect virgins of the required genotypes and set the cross. Typically, the teaching assistant amplifies the required stocks to hasten the progress of this step.

On an average the ratio of females to males per cross is 8:6. For the next two weeks, the students regularly flip the flies and incubate them at room temperature until they have about 5 tubes with healthy cultures growing in them. During the third week, the students observe the progeny under dissection microscopes.

Table 1.

Week 1:
(1) Learn to distinguish between male and female flies. Observe fly anatomy.
(2) Set crosses (below), and transfer to fresh food vials
  (a) GMR GAL4 females × UAS *Hpo* males
  (b) bi GAL 4 females × UAS *Ey* males

Week 2: Flip flies into fresh food vials
Week 3: Observe phenotypes of the progeny in F1 generation, score phenotypes, estimate ratios of flies that are wild-type versus the ones showing phenotype, and document effects by taking images of the adult flies in an apotome.

The students record the following data points:

1. Counts from the F1 progeny for each phenotypic class.
2. Are all expected classes observed; is there lethality, and if so what is the explanation for the observed lethality?
3. In case of the F1 progeny from the GMRGAL4 flies crossed to UAS-Hpo flies, pharate lethals are observed. The students are shown how to dissect the pharates and observe the ectopic eye phenotype.
4. The data for each experiment are recorded by taking images of adult flies (Figure 2) with the over-expression phenotype.

Conclusions

We have successfully carried out this laboratory exercise for three semesters now. The students have shown interest and appreciated how genes influence pattern or cell fate determination through the bi-GAL4 UAS-Ey experiment (Figure 2a, b). The cytotoxic effects of Hpo over-

expression (GMR-GAL4 UAS-Hpo) also illustrate the effect of normal *versus* abnormally high levels of protein expression in a cell (Figure 2c, d). The stark contrast in the outcomes of the two experiments illustrates the importance of normal regulation of genes during development and how mis-expression or over-expression of genes alters the pattern or causes inappropriate patterning. At the end of this exercise students learn about the mechanisms of regulation of gene expression, specifically the role of different promoters in generating tissue-specific gene expression profile along the temporal axis during development. They also study the role of transcription factors, DNA binding sites, and the effects of mis-expression of genes. These exercises provide students the hands-on experience that align theoretical knowledge with actual wet-bench experimentation and reinforce the concepts learned in class.

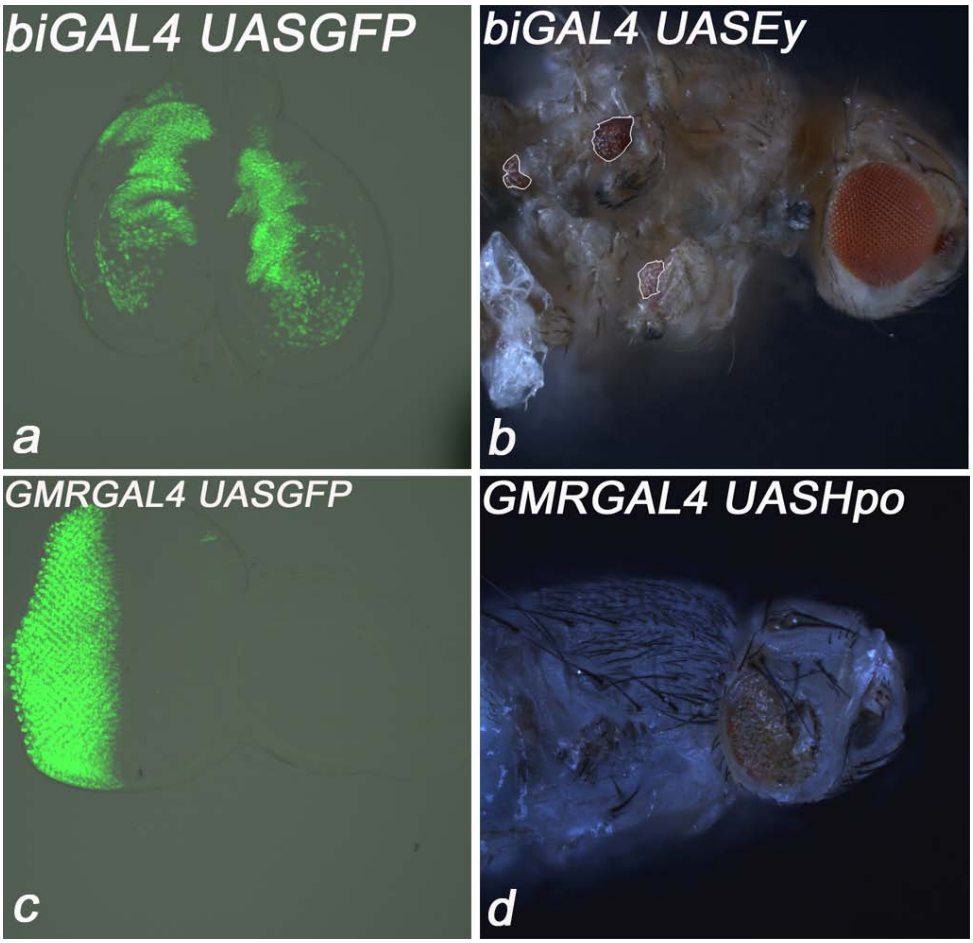

Figure 2. Effects of mis-expression of Ey and Hpo: a) Over-expression of UAS-GFP using bi-GAL4. GFP expression (green) is observed in the dorsal domain the leg and haltere disc. b) Over-expression of UAS-Ey using bi-GAL4 produces ectopic eyes on legs, wings, and halteres (marked by white lines). c) Over-expression of UAS-GFP using GMR-GAL4. GFP expression (green) is seen in the photoreceptor neurons. d) Over-expression of UAS-Hpo using GMR-GAL4 produces small necrotic eyes in the adult.

References: Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Halder, G., P. Callaerts, and W.J. Gehring 1995, Science 267: 1788-1792; Kango-Singh, M., A. Singh, and Y. Henry Sun 2003, Dev. Biol. 256: 49-60; Phelps, C.B., and A.H. Brand 1998, Methods 14: 367-379; Tare, M., O.R. Puli, M.T. Moran, M. Kango-Singh, and A. Singh 2012, Genesis; Udan, R.S., M. Kango-Singh, R. Nolo, C. Tao, and G. Halder 2003, Nat. Cell Biol. Oct, 5(10): 914-920; Verghese, S., S. Bedi, and M. Kango-Singh 2012, Cell Death Differ. Oct, 19(10): 1664-1676.