#### **DETECTION OF GENE ENHANCERS BY Drosophila ENHANCER TRAP ANALYSIS**

**Enhancers:** Transcriptional enhancers are DNA sequences that control expression of nearby genes and direct tissue-specific, positive regulation of those genes (Walker & Rapley,1997; also, any contemporary genetics or cell biology textbook). The general mode of action of these sequences is that they associate with specific activator proteins (that are found in only certain tissues); this association of enhancer and activator facilitates transcription initiation at the target gene's promoter. Often located 1 kb or more from the target promoter, enhancers might be located upstream, downstream or even within the transcription unit that they control. Under the influence of one or more enhancers, a gene can be abundantly expressed in certain tissues of the organism (*i.e.*, cells in which the activator protein is found) and weakly or not at all expressed in other tissues. By identifying and studying enhancers, we learn how genes are regulated in time and space within multicellular organisms and we are able to use enhancers to "tag" tissues or even individual cells.

**Enhancer trap strategy**: A genome-wide method for detecting and studying enhancers has been developed called enhancer trap mutagenesis. This method utilizes a recombinant transposon construct that can be inserted at essentially random sites within the genome and that, when inserted near an enhancer, will express a reporter gene product within the tissue or cells containing activator protein for that enhancer. The enhancer trap transposon contains a "basal" promoter element linked to a reporter gene that is inactive, except when influenced by a nearby enhancer. Because most enhancer usually results in reporter gene expression, insertion of the enhancer trap transposon near an enhancer normally functions. By mobilizing the transposon to diverse, essentially random chromosomal sites, one can detect diverse enhancers at those sites and study the tissue-specific gene from genomic DNA adjacent to the site of any particular insertion, enabling the isolation of the enhancer and its target gene from genomic DNA. This has proved to be a powerful system for studying enhancers and genes that direct highly specific cell expression (*e.g.*, genes that are expressed in individual cells that direct or reflect differentiation of those cells and their neighbors).

**Specific transposon:** The P[*lacW*] transposon (Fig. 1) consists of the open reading frame (ORF) for *E. coli lacZ* joined to the 5' end of a P element, such that the P second exon and the *lacZ* ORF are fused inframe (Bier, *et al.*, 1989). The resulting protein includes the first 123 amino acids of the P protein joined to the *lacZ* ORF. Expression of this fusion protein is controlled by the P element promoter that is expressed very weakly in all tissues, unless a nearby enhancer invokes abundant expression. The fusion protein is relatively unstable, permitting detection of both temporal and spatial control by the enhancer. P[*lacW*] also contains *Hsp-w*<sup>+</sup> sequences, a *Hsp70* promoter-*white*<sup>+</sup> gene fusion that provides a dominant  $w^+$  marker with which we can detect the element in flies, and bacterial plasmid vector sequences. All of these sequences are located within inverted copies of the P element 31 bp inverted repeat sequences (denoted as "squiggles" in Fig. 1) that will permit mobilization of the transposon within cells containing the necessary P element transposition protein called transposase.



**DET Fig 1**. The P[*lacW*] transposon (Bier, *et al.*, 1989). Extents of the *lacZ* and *Hsp-w* genes, the bacterial plasmid (BS), and P element terminal repeat sequences are indicated.

**E. coli lacZ as a reporter gene**: *lacZ* gene fusions are widely employed as reporters to measure transcriptional and translational regulation. *E. coli*  $\beta$ -galactosidase is an inherently stable protein and, as a foreign protein, it appears to lack signals borne by most eukaryotic proteins that govern their processing and longevity in eukaryotic cells. Differences in  $\beta$ -galactosidase activity in different tissues of an animal are reflections of rates of synthesis of the reporter protein in those tissues, which in turn is usually determined by the abundance of reporter mRNA. For a wide range of cellular systems, transgene  $\beta$ -galactosidase enzymatic activity is proportional to rates of transcription of the transgene copy. Another advantage of *lacZ* reporter systems is the highly sensitive and versatile array of detection techniques that have been devised during its long study and use in *E. coli*. In this exercise, you will use the artificial  $\beta$ -galactosidase substrate X-gal (5-bromo-4-chloro-indoly]-*D*-galactopyranoside) that, when hydrolyzed by the enzyme, produces a vivid blue dye. This provides a sensitive means of detecting and roughly quantitating reporter gene expression in whole tissue preparations (Glaser, *et al.*, 1986).

Each group will perform  $\beta$ -galactosidase staining of tissues from *D. melanogaster* adults and larvae of the strain that you have been studying this semester; the P[*lacW*] transposon is inserted at a single site in your strain, but the site differs in strains studied by diverse groups. Because these strains differ in their insertion sites and, therefore, expression of the weak P element promoter within the P[*lacW*] transposon might be influenced by novel enhancer elements, it will be necessary to stain a variety of tissues in each strain to ascertain the type(s) of enhancers effective in each strain.

# β-Galactosidase Staining

**1.** Perform  $\beta$ -galactosidase staining of adult abdominal tissues. For dissecting and staining flies (and their viscera), you will need a dissecting microscope, a pair of watchmaker forceps, two depression dishes, a heat block set at 37°, microscope slides, coverslips and the solutions described below.

**2.** Anaesthetize some adult animals and observe them under the dissecting microscope. All should have orange-to-red eyes. They may have dominant markers *Cy* (*Curly* wing) or *Sb* (*Stubble* bristles), indicating that the insertion is maintained in a heterozygous stock (probably recessive lethal).

**3.** Dissect and stain animals in a series of steps (modified from Glaser, *et al.*, 1986). First, place 400  $\mu$ l Buffer A in a well of a depression dish and dissect an anesthetized fly directly in the fixative. Dissect up to three males and three females in the same well, discarding the carcasses.

Buffer A:	10 mM NaPO <sub>4</sub> , pH 7.5 150 mM NaCl 1 mM MgCl <sub>2</sub>
Fixative:	0.25% glutaraldehyde in Buffer A Add 12.5 μl 70% glutaraldehyde to 3.45 ml Buffer A

#### Avoid contact with glutaraldehyde and avoid inhaling its fumes.

**4.** After dissecting all animals, transfer all of the viscera to 200  $\mu$ l fixative in another well of the depression dish. Allow the sample to fix for 5 minutes, then transfer the tissue to the remaining well of the depression dish containing 400  $\mu$ l Buffer A. This washes away the fixative and hold the tissues until you have dissected all of your samples.

**5.** Find several large larvae that are "wandering" around the wall of your culture vial (these are third instar larvae that are seeking a place to pupariate). Collect them with your forceps and wash by dipping in a pool of Buffer A in another depression dish. Dissect and stain these animals directly in the fixative, following the instructions given in class and the protocol in the above step #4.

**6.** After you have completed dissecting and fixing organs from your line, draw off the Buffer A from each well and replace it with 200  $\mu$ l of stain mix.

Stain Mix:1.55 ml McIlvaine buffer (0.1 M NaPO4 to pH 8 with<br/>citric acid)0.2 ml 50 mM K3Fe(CN)6<br/>0.2 ml 50 mM K4Fe(CN)6

Warm to 37° in a heat block Add 50 : I 8% X-gal (in N,N-dimethylformamide) Maintain at 37° to avoid X-gal precipitation

**7.** Place the dish in a sealed, humid box. Incubate at  $37^{\circ}C$  overnight, to permit  $\beta$ -galactosidase staining to proceed.

**8.** To stop the staining, remove the stain mix from the well, removing as many X-gal crystals as possible with the stain mix. Replace the stain mix with Buffer A and return the depression dish to the sealed, humid chamber while the crystals attached to the tissue fragments dissolves. *This will be done by the instructors on the day after the lab and the samples will be stored at 4°C until the next lab.* 

# Mounting and Observing Stained Tissues

**9.** Transfer the stained adult tissue to a drop of mounting medium on a new microscope slide. Place all tissues from all animals onto a single slide, preparing one slide for adult tissues and one slide for larval tissues.

# Mounting Medium: 50% glycerol in Buffer A

**10.** Gently overlay the drop with a new coverslip. Under the microscope, gently press the coverslip over the tissue to splay tissues for observation.

**11.** Slides may be stored in a moist, sealed box and re-examined for several weeks, without deterioration of the tissues or fading of the staining pattern.

**12.** Observe the staining pattern in tissues of each line. Use the provided anatomical diagrams to determine which tissues express  $\beta$ -galactosidase. Record your observations.

#### References

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