

GENOMICS ARTICLE

Ten Years of Enhancer Detection: Lessons from the Fly

Hugo J. Bellen¹

Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Program in Developmental Biology, Baylor College of Medicine, Houston, Texas

INTRODUCTION

Traditional genetic screens typically have focused on genes that affect specific phenotypes. These screens, therefore, often have failed to identify genes whose functions overlap or are limited to specific phases of development, or that are expressed in a limited number of tissues. In addition, sequence analyses of whole genomes have revealed that traditional mutant screens have assigned functions to only a minority of a given organism's genes. An important alternative to mutant analysis in investigating gene functions is offered through an investigation of expression patterns. To this end, transposon-based enhancer detection has been used successfully in *Drosophila*. This approach is known colloquially as enhancer trapping, but the preferred term is enhancer detection, because the reporter construct is not actually trapped by enhancers but rather is integrated into genomic sequences to promote the detection of adjacent enhancers.

Significant progress has been made in enhancer detection in *Drosophila* over the past 10 years through the use of *P* elements. Enhancer detection and related methodologies not only have provided a successful means of identifying new genes but also have been the backbone for the creation of many new tools in *Drosophila* biology, permitting sophisticated genetic manipulation with great ease. Here, I review developments in methodology in an attempt to suggest how these approaches may be applicable to other species, including plants. The concept that expression patterns can be studied to assign gene function is broadly accepted, as is reflected in the current popularity of microarray-based expression analyses. Unfortunately, microarray-based approaches suffer from limited resolution, so they do not offer a direct means of monitoring or manipulating the expression of specific genes within a single cell or small group of cells. Enhancer detection and associated methods, therefore, will be integral to the "toolbox" of functional genomics. Indeed,

many enhancer detection and related technologies are now being used in genomewide surveys, producing large data sets on gene expression that can be compiled into genomic databases. Hence, these new methods not only facilitate the identification, isolation, and characterization of specific genes but also are contributing to the rapidly developing field of functional genomics.

HISTORY AND PRINCIPLES

Gene enhancer detection was first developed for use in bacteria by Casadaban and Cohen (1979), who designed a vector that allowed random integration of a Mu bacteriophage carrying a promoterless *lac* operon into the *Escherichia coli* chromosome (Figure 1A). Activation of the *lac* reporter gene required integration of the phage downstream of a bacterial promoter in the proper orientation (Casadaban and Cohen, 1979). Whereas insertions into constitutively expressed genes led to constitutive *lacZ* expression, insertions into genes whose expression was regulated led to *lacZ* induction only under specific conditions, such as those resulting from DNA damage. The method was attractive because it allowed screens for specific classes of response genes that were simultaneously tagged, thereby permitting rapid cloning. These two important advantages prompted others to extend the methodology to other bacterial systems, such as *Bacillus subtilis* (O'Kane et al., 1986).

It was almost a decade later that the principles of enhancer detection established in *E. coli* were applied to multicellular eukaryotes. Inasmuch as eukaryotic genomes are less densely occupied by genes than are prokaryotic genomes, they contain sequences that can regulate promoters at considerable distances (Wilson et al., 1990). It therefore was not surprising that reporter constructs containing minimal promoters (instead of the promoterless reporters used in the bacterial systems) integrated into the eukaryotic genome to reveal the complexities of expression patterns more effectively (O'Kane and Gehring, 1987; Figure 1B).

¹To whom correspondence should be addressed. E-mail hbellen@bcm.tmc.edu; fax 713-798-8515.

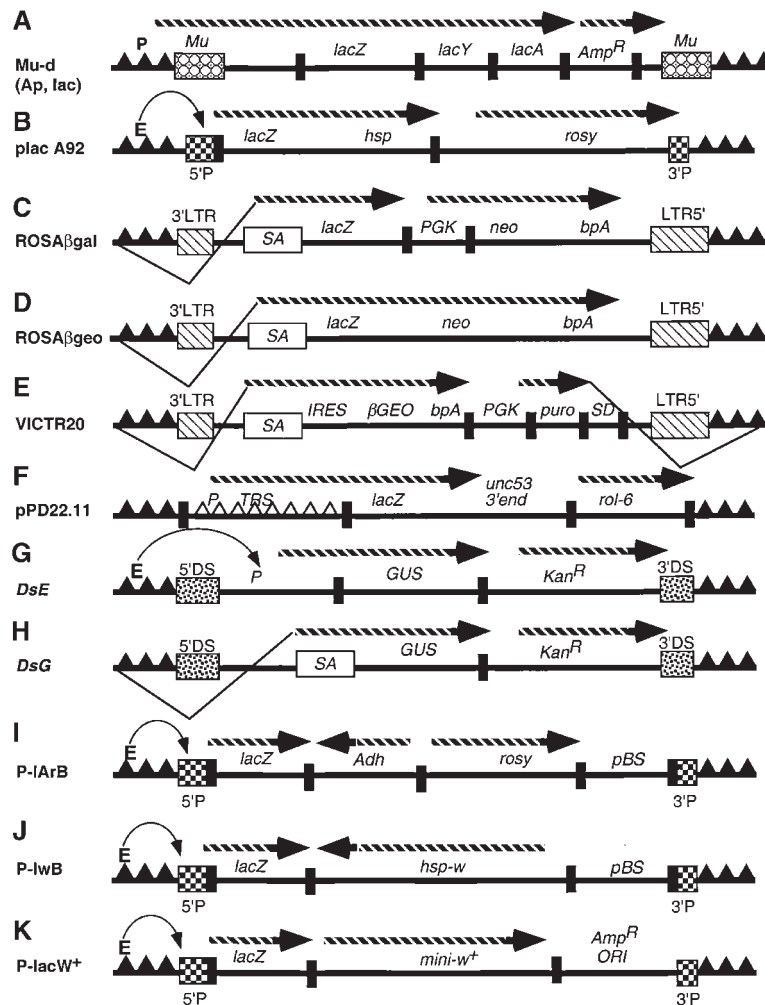


Figure 1. Gene Enhancer Detectors and Gene Traps Used in the Past 10 Years.

Promoter traps require endogenous promoters (P) ([A] and [F]). Enhancer detectors capitalize on the presence of nearby genomic enhancers (E) ([B], [G], and [I] to [K]).

Gene traps contain splice acceptor (SA) sites to produce fusions to the reporter gene ([C] to [E] and [H]).

(A) The Mu bacteriophage construct first used in *E. coli*. *lacZ*, *lacY*, and *lacA* form the bacterial *lac* operon.

(B) The first *P*-element enhancer detector used in *Drosophila*.

(C) By providing an SA site at the 5' end of *lacZ*, retroviral insertions may produce fusion proteins.

(D) Similar to (C), but the fusion protein (β geo) consists of the endogenous protein fused to both β -galactosidase and neomycin.

(E) The advantages of *ROSA* β gal (C) and *ROSA* β geo (D) are combined into one vector.

(F) A construct used in *C. elegans* as a promoter trap for genomic fragments inserted into a transformation vector.

(G) Enhancer detector used in *Arabidopsis*.

(H) Gene trap used in *Arabidopsis*.

(I) Second-generation enhancer detector used in *Drosophila*.

(J) Similar to P-IArB (I).

(K) Commonly used enhancer detector in *Drosophila*.

Adh, alcohol dehydrogenase gene, which can be used as a positive and negative selectable marker; *Amp*^R, ampicillin resistance; *bpA*, poly(A) addition site; *GUS*, β -glucuronidase; *hsp*, heat shock protein gene poly(A) addition site; *hsp-w*, white gene of *Drosophila* used to identify transformants; *IRES*, internal ribosomal entry site; *Kan*^R, kanamycin resistance; LTR, long terminal repeat of retrovirus; *mini-w*⁺, a white gene of *Drosophila*; *neo*, neomycin resistance gene; ORI, origin of replication of *E. coli*; pBS, Bluescript vector; *PGK*, a constitutive promoter expressed in all cells; *puro*, puromycin resistance gene; *rol-6*, a dominant marker to identify transformants; *rosy*, eye color marker to identify transformants; SD, splice donor site; *TRS*, translation initiation site; *unc53* 3'end, a poly(A) addition site.

Because many eukaryotic enhancers act in an orientation-independent fashion, activation of a reporter construct does not normally require insertion downstream from the transcription start site of a genome-endogenous promoter. Similarly, the orientation of insertion is often irrelevant.

An important question is how faithfully does the expression pattern of the enhancer-detecting insert reflect the expression pattern of the endogenous gene? A survey of the *Drosophila* literature shows that in most cases, the enhancer detector pattern mimics the endogenous pattern, and that expression is generally confined to the subset of tissues or cells that express the endogenous gene (Kania et al., 1993; McCall et al., 1994; Casares et al., 1997). This observation thus corroborates the modular nature of eukaryotic enhancers (Wilson et al., 1990). In fact, it is rare that enhancer detector expression occurs in cells or tissues in which the endogenous gene is not normally expressed. However, when β -galactosidase is used as a reporter, expression often is prolonged relative to expression of the endogenous gene, especially when a bipartite system is used (see below).

Enhancer-detecting reporter constructs represent powerful tools not only for isolating novel genes but also for implementing cell- and tissue-specific markers, determining lineages of organs and cells, ablating specific cells, and marking chromosomes for subsequent genetic studies. Moreover, the methodology developed in prokaryotes and *Drosophila* has been adapted by biologists working in a wide variety of systems and species.

BEYOND THE FRUIT FLY

Studies in the Mouse

Mouse geneticists have placed emphasis not on enhancer detectors per se but rather on promoter and gene traps in which reporter constructs are trapped downstream of a promoter or within a coding region (reviewed in Rossant and Hopkins, 1992). Many different mouse gene trap vectors have been constructed. Those that are most often used (Figures 1C and 1D) share three basic features: (1) they are derived from retroviruses and hence have long terminal repeats; (2) they contain a splice acceptor (SA) site permitting splicing of the *lacZ* coding sequence to exons of endogenous genes, obviating the need to insert into exonic sequences; and (3) they contain a positive selectable marker (neomycin or puromycin resistance) that either is under the control of a constitutively active promoter or is fused directly to the *lacZ* gene (see Figures 1C and 1D; Gossler et al., 1989; Kerr et al., 1989; Friedrich and Soriano, 1991; Rossant and Hopkins, 1992). Insertions of such constructs into embryonic stem (ES) cells, from which whole animals can be regenerated via blastocyst injection or morula aggregation, allow analyses of gene expression patterns and sometimes permit the study of mutants arising from intragenic disrupt-

tions. Indeed, \sim 30% of insertions in one study resulted in lethal mutations (Friedrich and Soriano, 1991).

The gene trapping strategy in mice recently has been advanced using a more sophisticated vector, VICTR20 (Figure 1E), along with automated screening processes (Zambrowicz et al., 1998). Upon infection of ES cells with the vector, between 30,000 and 40,000 ES cell clones were created such that each contained a single insertion. Because of the nature of the vector, with both SA and splice donor sites, the selectable marker is activated only if properly spliced between adjacent exons of an endogenous gene. The exonic sequences, which are identified easily by reverse transcription-polymerase chain reaction (PCR) and subsequent sequencing, have been deposited in the Omnibank database (<http://www.lexgen.com/OmniBank.html>) as Omnibank sequence tags. Thus, when investigators identify a gene of interest, for a fee they can search the commercial Omnibank sequence tag database, and the corresponding ES cell clone with a specific retroviral insertion can be purchased for microinjection into host blastocysts to produce mutant mice. This approach is an important step forward in mouse genomics. Unfortunately, the cost of obtaining the desired ES clones is prohibitive for many investigators, which emphasizes the need for a similar, noncommercial database. An additional limitation to the use of the approach arises in that the mutagenicity of the vector was not fully investigated (Zambrowicz et al., 1998), raising the possibility that the VICTR20 retrotransposon may not be as mutagenic as those previously described by Friedrich and Soriano (1991). A further concern for these approaches is that retroviral sequences may have polar effects and thus affect expression of adjacent genes.

Studies in *Caenorhabditis elegans*

In the nematode *C. elegans*, only a modified version of promoter trapping has been used to detect expression patterns. Transposable elements cannot be manipulated easily in *C. elegans* because transposase expression in germline cells cannot be properly controlled. Similarly, DNA-mediated transformation normally creates extrachromosomal arrays rather than integration into genomic DNA. As an alternative to enhancer detection or gene trapping, genomic DNA fragments were cloned into a transformation vector (pPD22.11; Figure 1F) upstream of the *lacZ* gene (Hope, 1991). Because this strategy requires a transcriptionally and translationally productive fusion with the *lacZ* gene in the vector, it can be considered a variant of promoter trapping. Upon transformation, \sim 1% of all genomic fragments tested conferred β -galactosidase expression. Because the entire *C. elegans* genome has been sequenced, the genomic promoter responsible for expression of the reporter gene can be readily identified. The scale to which these experiments have been carried has defined many enhancer-promoter fragments, and such data are being integrated into the *C. elegans*

AceDB database (<http://elegans.swmed.edu>; Lynch et al., 1995; Hope et al., 1996).

Studies in Arabidopsis

In plants, enhancer detection and gene trapping via a transposon system were introduced by Sundaresan et al. (1995) and Klimyuk et al. (1995). Most of these vectors, two of which are shown in Figures 1G and 1H, exploit the heterologous maize *Ds* transposon system in Arabidopsis (Aarts et al., 1993; Bancroft and Dean, 1993; Long et al., 1993). Approximately one-half to two-thirds of the enhancer detector insertions (Klimyuk et al., 1995; Sundaresan et al., 1995) and one-quarter of the gene trap insertions (Sundaresan et al., 1995) resulted in expression of the reporter gene in seedlings or flowers. Several of these transposition events proved to be mutagenic and led to the identification of new genes (Aarts et al., 1993; Gu et al., 1998). It appears, however, that this methodology still is not being used to its full potential in Arabidopsis, because the literature contains few examples of genes identified by this technique.

GENERATION AND USE OF CELL-SPECIFIC AND TISSUE-SPECIFIC MARKERS IN DROSOPHILA

In *Drosophila*, the first enhancer detectors were constructed from transposable *P* elements (O'Kane and Gehring, 1987; Figure 1B) because *P*-element transformation can be very efficient (Spradling and Rubin, 1982). Vectors that allow rapid cloning of the DNA flanking the *P*-element insertion site, via plasmid rescue, were developed later (Figures 1I to 1K; Bier et al., 1989; Wilson et al., 1989). The transposase gene promoter is only 87 nucleotides from the 5' end of the *P* element and thereby appears to be readily accessible to nearby enhancers, which then elevate transcription levels above otherwise very low basal rates in most genomic integration sites (O'Kane and Gehring, 1987). The first *P*-element enhancer detector screens were greatly facilitated by the implementation of an endogenous nonmobile source of transposase (Cooley et al., 1988; Robertson et al., 1988). Specifically, the nonmobile transposase source allowed the transpositional creation of thousands of strains from a single parental *P*-element strain.

Perhaps the most surprising finding of the early screens was the high efficiency of the enhancer detector methodology in detecting expression patterns. Indeed, the majority of strains that carried a single enhancer detector insertion expressed the β -galactosidase reporter (Bellen et al., 1989; Bier et al., 1989; Perrimon et al., 1991). The simplest explanation for this high frequency of expression is that *P* elements preferentially insert into the 5' regulatory regions of genes, as observed for many transposable elements in other species (Bellen et al., 1992a; Grossniklaus et al., 1992; Eldon et al.,

1994; Engels, 1996; Parinov et al., 1999, in this issue). The 5' regulatory region of genes is probably the ideal position for enhancer detection vectors to "pick up" the activity of enhancers. This fortuitous fact is possibly the most important reason for the success of this methodology in *Drosophila*.

The expression patterns evident in the early enhancer detection studies showed that 5 to 10% of the strains with single *P*-element insertions expressed the reporter in very specific tissues or cells, thus providing many excellent new tissue and cell markers (Bellen et al., 1989; Bier et al., 1989; Hartenstein and Jan, 1992). The β -galactosidase expressed in the early enhancer detection strains was localized to the nucleus (O'Kane and Gehring, 1987); markers have since been designed to label cellular projections.

Because any given enhancer will not necessarily act equally well with all minimal promoters, those other than that of *P*-element transposase have been evaluated as well. In two studies, minimal promoters from genes that control segmentation in the early embryo were found to confer a higher frequency of tissue-specific reporter expression and skew the expression patterns to other tissues and cells compared with patterns established with the *P*-element promoter (Perrimon et al., 1991; Mlodzik and Hiromi, 1992). For example, a promoter from a segmentation gene that is expressed in vertical stripes in the embryo promoted striped patterns, probably due to the presence of weak "stripe" enhancers contained within the promoter. These data suggest that the use of multiple promoters is advisable in enhancer detection screens. Furthermore, these screens used a cytoplasmically localized β -galactosidase, showing that nuclear localization was not required for efficient detection of β -galactosidase expression.

Expression of cytoplasmic β -galactosidase is, however, too weak in most enhancer detector strains to label thin cellular processes, such as axons and dendrites, which is a major limitation to analyses of neuronal connectivity, growth cone guidance, and target recognition in the nervous system. A kinesin-*lacZ* enhancer detector was constructed (Giniger et al., 1993) to transport β -galactosidase along cellular processes such as axons. Similarly, a tau- β -galactosidase fusion that is transported to axons and synapses has been designed (Callahan and Thomas, 1994). These enhancer detectors label processes of nonneuronal cells in great detail, providing a more refined tool to study processes such as muscle attachment. More recently, many *Drosophila* researchers have explored the usefulness of the green fluorescent protein (GFP) or its enhanced versions to monitor cells in the living organism (Brand and Dormand, 1995). Some strategies using GFP have proven quite successful, and their use is becoming routine.

One of the major applications of enhancer detection has been in the implementation of cell and tissue markers in systems that previously had been intractable (O'Kane and Gehring, 1987; Bellen et al., 1989; Bier et al., 1989). In this way, enhancer detectors have established the origin of many cells and tissues, thereby extending our knowledge of embryogenesis and organogenesis. The impact of the methodology

is best illustrated by comparing the first and second editions of the standard work "The Embryonic Development of *Drosophila melanogaster*" (Campos-Ortega and Hartenstein, 1985, 1997). The latter edition contains more than 60 photographs of enhancer detector strains labeling specific cells in embryos. Indeed, enhancer detector strains have helped in establishing cellular origins and lineages in countless tissues and organ systems, including the peripheral nervous system (Bier et al., 1989; Ghysen and O'Kane, 1989; Bellen et al., 1992c; Forjanic et al., 1997), the central nervous system (Klambt et al., 1991; Datta et al., 1993; Davis and Han, 1996), glial cells (Klambt et al., 1991), hemocytes (Rodríguez et al., 1996; Braun et al., 1997), fat body (Hoshizaki et al., 1995), gut (Murakami et al., 1994), imaginal discs (Gibson and Gehring, 1991; Brook et al., 1993), tracheal lineages (Samakovlis et al., 1996), and gonads (Grossniklaus et al., 1989; Gonczy et al., 1992). These studies have led to the creation of a rich library of strains that express *lacZ* in specific cells or tissues and that can be used for a wide variety of applications.

ENHANCER DETECTORS AS MUTAGENS

The efficiency with which *P* elements cause mutations is poor when compared with that of chemical mutagens such as ethyl methanesulfonate (EMS) or ethyl nitrosurea (Ashburner, 1990). Hence, *P*-element mutagenesis screens are much more labor intensive than chemical screens. Indeed, only 11 to 13% of all *P*-element insertions cause a visible or lethal phenotype (for discussion, see Roseman et al., 1995). In addition, *P* elements tend to insert into hot spots (Bellen et al., 1992a; Kania et al., 1995; Salzberg et al., 1997), often restricting the rate of insertions at unique loci to a mere 5 to 7%. Nevertheless, *P* elements remain the mutagen of choice in *Drosophila* for several reasons. First, sequences adjacent to *P* elements can be easily cloned using inverse PCR and/or plasmid rescue (Wilson et al., 1989), two methods that are efficient and rapid. Second, *P*-element insertions can be easily mapped to chromosomal regions by in situ hybridization of polytene chromosomes. This methodology, however, soon will be outdated because mapping is increasingly accomplished through the sequencing of inverse PCR products followed by comparison genome sequences available in the public database. Third, *P* elements can be excised in the presence of a stable transposase (Cooley et al., 1988; Robertson et al., 1988). Precise excision that can be correlated to reversion of the phenotype uniquely confirms the given insertional mutation as the direct cause of the phenotype. Finally, imprecise excision allows the generation of new alleles that can become valuable in phenotypic analysis. For these reasons, *P*-element insertions have become an integral component of the *Drosophila* genome project (Spradling et al., 1995, 1999). The most interesting *P*-element insertional mutants to be recovered in enhancer detec-

tor screens have been assimilated at the Bloomington Stock Center and the European Stock Center in Hungary (Cooley et al., 1988; Bellen et al., 1989; Bier et al., 1989; Gaul et al., 1992; Chang et al., 1993; Torok et al., 1993; Deak et al., 1997; Spradling et al., 1999). These *P*-element-containing strains have aided in the isolation and characterization of numerous genes and are freely available to the scientific community.

In a screen for *P*-element insertional mutations, potentially interesting strains may be selected through two fundamentally different approaches: observation of the expression patterns arising from enhancer detection and phenotype analysis. In smaller screens, one often uses both criteria simultaneously, whereas in large screens, like those conducted by Torok et al. (1993) and Deak et al. (1997), lethal or semilethal insertions are initially chosen and subsequently screened for β -galactosidase expression. Note that phenotype analysis can identify strains for further study that expression pattern analysis does not, and vice versa. For example, Kania et al. (1995) and Salzberg et al. (1997) screened 4500 homozygous lethal strains (Deak et al., 1997) for a visible phenotype, namely, altered patterning of the embryonic peripheral nervous system as revealed by staining with a neuronal-specific antibody. The same strains were independently stained for β -galactosidase activity in the nervous system. Interestingly, although each screening technique identified >200 potentially interesting strains, only a few strains (<10) were identified by both methods. These data show that *P*-element-disclosed expression patterns do not necessarily correlate with morphological defects (see also Kania et al., 1995; Salzberg et al., 1997). The explanations for discrepancies between expression patterns and phenotypes may lie in any of three possibilities. The first is that *P* elements associated with a severe phenotype can be inserted within genes that are not expressed in a temporally and spatially restricted pattern. The second is that *P* elements associated with a severe phenotype may be inserted into the coding region of genes, where they are less likely to be influenced by nearby enhancers. Conversely, *P* elements that do not cause obvious phenotypes may be inserted upstream of transcription start sites, where they do not disrupt gene function but are better able to respond to nearby enhancers. Hence, it is recommended that both phenotypic and expression screens be performed in parallel, regardless of the particular species under study.

Another issue is the difference in mutagenicity between *P* elements and chemical mutagens such as EMS. Many investigators decide not to pursue EMS mutagenesis if they succeed in isolating a *P*-element insertion within their gene of interest. However, *P*-element mutagenesis is no substitute for EMS mutagenesis (Salzberg et al., 1997). First, *P* elements tend to insert in a nonrandom fashion, which may preclude mutagenesis in all of the genes involved in a specific biological pathway. Indeed, each chromosome seems to contain 5 to 10 "hot spot" genes that accumulate as many as 30 to 50% of the *P*-element insertions in any

particular screen (for examples, see Kania et al., 1995; Salzberg et al., 1997; Spradling et al., 1999). However, Spradling et al. (1999) have estimated that mutations in as many as 85% of all genes can be recovered if enough *P*-element insertions are screened. Second, *P*-element inserts tend to create regulatory mutations that affect expression levels and cause only partial loss of gene function (Bellen et al., 1992a; Schulze et al., 1995; Grotewiel et al., 1998). On the other hand, EMS mutations tend to affect single nucleotides in coding regions, thereby creating nonsense or missense point mutations that can provide structure–function information about the gene product. Hence, EMS-induced alleles are often more severe than *P*-element–induced mutations (Salzberg et al., 1994, 1997). Obviously, imprecise excisions of *P* elements can create null alleles of the gene adjacent to the insertion site. However, these null mutations tend to reflect the loss of regulatory functions rather than amino acid substitutions.

Given the general insertional specificity of *P* elements, it has been difficult to predict the percentage of genes that potentially can be mutated. Indeed, estimates vary from 50 (Kidwell, 1987) to 85% (Spradling et al., 1999). Two methods have been considered to sample a broader set of genes than those hit by *P* elements. Smith et al. (1993) designed a new enhancer detector system based on *hobo* transposable elements. They reported that the *hobo* system was less efficient than many *P*-element enhancer detection systems in revealing the expression patterns, but they nevertheless provided good evidence that insertional specificities of the two systems differed significantly.

A complementary strategy aimed at sampling a broader set of genes is to exploit the local intrachromosomal “hopping” of existing *P*-element insertions (Tower et al., 1993; Zhang and Spradling, 1993). The transposition of a *P* element into a nearby known sequence can be monitored by PCR (Littleton et al., 1993). Alternatively, transposition into a gene of unknown sequence can be monitored by complementation analysis, provided that appropriate mutants have been previously generated (Dye et al., 1998). However, one cannot always recover insertions into a particular gene of interest by local transposition. For example, we have failed to obtain *P*-element insertions into the gene of interest in two out of four trials, even though several thousand transpositional events were screened in each trial (T. Lloyd, M. Wu, and H.J. Bellen, unpublished data). In addition, mutations arising from the imprecise excision of the source *P* element are obtained more often than the desired local transpositions (Bhat et al., 1999).

MANIPULATING GENE EXPRESSION, CELL VIABILITY, AND CELL FUNCTION

Several powerful methods for altering cell function have been developed in recent years, many of which have been

used in conjunction with enhancer detection methodology. These methods include the use of the bipartite upstream activating sequence–GAL4 (UAS–GAL4) system to alter specific gene expression or to express toxins that compromise or ablate cells or tissues (Kunes and Steller, 1991; Bellen et al., 1992b; Moffat et al., 1992; Brand and Perrimon, 1993; Sweeney et al., 1995; Phelps and Brand, 1998).

The UAS–GAL4 method is a bipartite system in which expression of the yeast *Gal4* gene is implemented within specific cells or tissues. The targeted delivery and subsequent expression of the GAL4 transcription factor can be achieved by substituting the *Gal4* gene for the *lacZ* gene in the types of enhancer-detecting constructs described earlier in Figures 11 to 1K. The expression pattern of the GAL4 protein is then visualized by introducing a second *P* element from which the expression of *lacZ* (or some other reporter gene) is controlled by an UAS (Figure 2A); the GAL4 transcription factor binds to the UAS and activates *lacZ* expression. Flies that contain such modified enhancer detectors have been screened to develop strains that express GAL4 in specific patterns (Brand and Perrimon, 1993; Gustafson and Boulianne, 1996; Ito et al., 1997; Manseau et al., 1997; Tissot et al., 1997). This method is very versatile because it allows subsequent use of the GAL4 strains to express any protein in a spatially and temporally restricted fashion simply by introducing a second construct in which the gene of interest is placed downstream of the UAS sequence. In addition to determining the consequences of (over)expression of a gene, the method can be applied to specific cells and tissues to rescue mutations, express a toxin, or implement a marker protein, such as enhanced GFP, to name a few applications (Brand and Perrimon, 1993; Phelps and Brand, 1998).

Because many more strains that contain *lacZ*-based enhancer detectors are available than strains that bear *GAL4*-based detectors, a gene conversion method has been developed (Sepp and Auld, 1999). With this method, the *lacZ* gene in integrated enhancer detectors can be replaced with the *GAL4* gene. Although the method is not applicable to all *P* elements, it has been reported to be quite efficient and in principle can be applied to all enhancer detectors.

The UAS–GAL4 system also has been used to identify genes involved in patterning of adult structures by using a noninvasive marker, the *yellow* gene. Fruit flies that carry a *yellow*[−] mutation have yellow cuticle, bristles, and wings but normal viability. The *Drosophila yellow*⁺ gene was placed downstream of the UAS, and the resulting reporter construct was integrated into a large number of *yellow*[−] backgrounds that collectively contained *GAL4*-expressing *P* elements randomly dispersed throughout the genome (Calleja et al., 1996). Approximately 90% of the resulting strains that carry UAS–*yellow*⁺ enhancer detectors express the *yellow*⁺ gene in some adult structures, implying that the majority of *Drosophila* genes are expressed in imaginal cells that give rise to adult tissues (Calleja et al., 1996). The major advantage of this technique is that there is no need to stain embryos, larvae, or adults for β -galactosidase activity and that

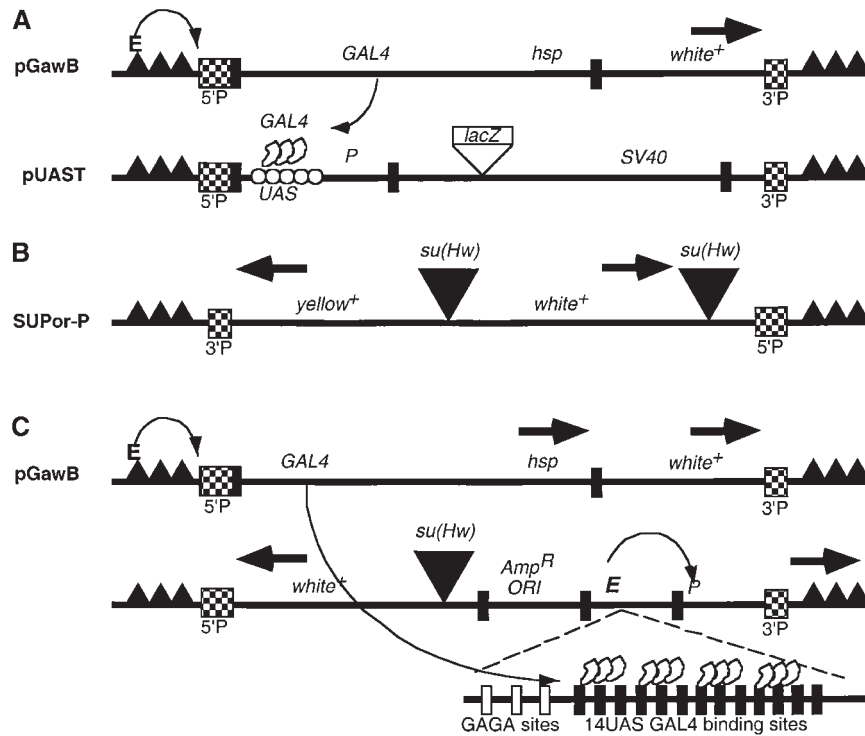


Figure 2. Other P-Element Vectors in Drosophila.

(A) The GAL4-UAS system relies on an enhancer detector that drives GAL4 expression in specific cells. The GAL4 protein then binds to UAS sites on a separate P element to activate another gene, such as the reporter gene *lacZ*. SV40, poly(A) addition site from SV40.
 (B) Because of the presence of *Su(Hw)* sites in this P element (see text), the mutagenesis efficiency is double that of most P elements. *yellow+* is body color marker.
 (C) A bipartite system can be used to direct misexpression of endogenous genes (see text). The first P element drives GAL4 expression in specific cells. GAL4 in turn activates a hybrid UAS-containing promoter on the second P element. The UAS-containing promoter faces outward and may lead to transcripts that include adjacent genes in the genome. Abbreviations are as given in the legend to Figure 1.

the patterns are fairly easy to detect. Although this method is similar to the UAS-GFP method (Brand and Dormand, 1995), it is simpler because there is no need for a UV light source. This methodology should be applicable to plants, by using, for example, the *R* gene, which conditions anthocyanin production (Lloyd et al., 1992).

Several methods have been developed to ablate specific cells by expressing the heterologous genes for toxins under the control of specific promoters/enhancers or using the bipartite UAS-GAL4 system. Strains that express GAL4 in specific cells or tissues have been used to drive two different types of toxin genes: diphtheria (Kunes and Steller, 1991; Bellen et al., 1992b) or ricin toxin (Moffat et al., 1992; Hidalgo and Brand, 1997). Both toxins interfere with protein synthesis, and temperature-sensitive mutants are available that also permit a temporal control of the ablation of cells (Bellen et al., 1992b; Moffat et al., 1992). In addition, a method that permits the functional ablation of neurons recently has been published. Tetanus toxin is known to cleave

synaptobrevin, a protein that is required for neurotransmitter release (Sweeney et al., 1995). By expressing the toxin in specific neuronal populations, one can abolish neurotransmitter release without interfering with the development of these cells.

ENHANCER SUPPRESSORS

As mentioned earlier, compared with chemical mutagenesis, P-element mutagenesis is inefficient in that lethal mutations are produced by only 12% of insertions, whereas EMS induces 60 to 80% lethal mutations per chromosome. Two strategies have been developed to interfere with endogenous enhancers to increase the mutational efficiency of P elements. The first acts to block the activity of enhancers by using a P element containing sequences that are bound by a protein called Suppressor of Hairy-wing (Su[Hw]) that blocks

the interaction of enhancers with endogenous promoters (Roseman et al., 1995; Figure 2B). Su(Hw) binding sites were originally discovered because they are required for the mutagenic activity of *gypsy* transposable elements (Corces and Geyer, 1991). Su(Hw) is expressed in most tissues throughout development (Harrison et al., 1993), and association of this protein with its binding site (present in wild-type *gypsy* element) prevents interaction of an enhancer with its promoter. Thus, insertion of a *gypsy* element between an enhancer and a promoter effectively allows Su(Hw) to block the enhancer–promoter interaction (Holdridge and Dorsett, 1991; Roseman et al., 1993). *P* elements containing Su(Hw) binding sites are particularly useful because most *P* elements insert into the 5′ regulatory sequences of genes. The modified *P* elements containing the Su(Hw) binding sites act as a more powerful mutagen than regular *P* elements, with 27% versus 12% of insertions causing lethality (Roseman et al., 1995).

In a second strategy, relying on the EP element, multiple UAS sites are integrated in front of a minimal promoter at the 3′ end of the *P* element (Figure 2C). Upon insertion, the *P* element permits ectopic expression of any genomic gene located at the 3′ end of the *P*-element insertion (Rorth, 1996; Figure 2C), thereby preventing access of any endogenous enhancer to its cognate promoter. As shown in Figure 2C, the *P* element contains, in addition to a Su(Hw) binding site, a total of 14 UAS sites upstream of a *Drosophila* promoter. After an initial set of EP insertions has been generated, EP-containing strains can be crossed to strains that express GAL4 in a specific cell or tissue, thus allowing misexpression of genes in cells or tissues specified by the GAL4-expressing strain. The GAL4 strain can be either an enhancer detector strain or a strain that expresses GAL4 from a cloned promoter (Rorth, 1996; Rorth et al., 1998). The advantage of this method is that it is conditional, even allowing screens for lethal-effect or sterile mutations. In addition, it is efficient because the same set of EP strains can be used to study the effects of misexpression in many different tissues, by simply crossing them to different GAL4 strains. Moreover, any loss-of-function phenotype caused by the EP element or its imprecise excision should be compared with the phenotype caused by the ectopic expression of genes induced upon insertion. The pitfall of this strategy is, of course, that over- or misexpression may cause nonspecific phenotypes that are not related to the normal function of the gene.

OTHER AVENUES

Another use of enhancer detectors is in phenotypic trapping (Pignoni et al., 1997). This method is based on replacing the *lacZ* gene in enhancer detectors with a cDNA encoding any given “protein X.” If protein X is expressed in the proper temporal and spatial patterns by virtue of the enhancer-

detecting activity of the construct, it will rescue the phenotype associated with “mutation X.”

Enhancer detectors also can be used in cell culture with Kc167 cells, where *P*-element transposition has been documented (Segal et al., 1996). Hence, it should be possible to generate millions of Kc167 cells, each of which contains a single or a few enhancer detectors. Using a marker for which both positive and negative selection is possible, one first can select against insertions in which the marker gene is constitutively expressed. In a second round, cells could be challenged with a physical insult such as heat shock, a chemical insult such as a drug, or a hormone. Using positive selection, one then can identify those cells in which the selectable marker is induced because of the treatment. Although this strategy may have little appeal to *Drosophila* geneticists, this approach could be envisaged in systems in which entire organisms can be regenerated from stem cells, such as in mice or plants.

SIGNIFICANCE TO GENOMICS

In the near future, the sequences of many genomes will be available. The next step will be to assign a function to every single gene. This challenge will be achieved by combining several methods that rely on genetics, biochemistry, and new technologies, such as expression microarrays and comparative genomics by using bioinformatics. It is likely that enhancer detectors and gene traps will play major roles in those organisms that are amenable to genetic analyses, such as *C. elegans*, *Drosophila*, mice, and plants, such as *Arabidopsis*. Comparable to ongoing work with T-DNA element and transposon insertions in *Arabidopsis*, the short-term goal in mouse and *Drosophila* biology is to obtain single gene traps and modified *P* elements near or in almost every gene. The advantage of using enhancer detectors and related schemes is that they may not only reveal considerable information about each gene but that they also can be used as tools to manipulate the pathways of interest.

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