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Enhancer Trap Technique: A Novel Tool for Identification and Developmental Characterization of Genes of Drosophila

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Enhancer trap technique – A novel tool for identification and developmental characterization of *Drosophila* genes

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The classical technique of mutational screen for identification of genes controlling early development has now approached saturation. A new era in genetic identification and developmental characterization of genes in *Drosophila* has commenced with the advent of the enhancer trap technique. This technique involves mobilization of a P *lacZ* vector to diverse chromosomal locations in the fruit fly genome to bring it under the regulation of developmentally expressed genes or their enhancer elements. The technique offers a strikingly elegant method of gaining entry into fruit fly genes.

DURING the development of the higher organisms, an apparently uniform egg cell gives rise to a complex metamerized adult. In the past decade and a half there have been dramatic improvements in our understanding of the principles governing the correct segregation of the developmental potential of cells in a growing embryo in a well-defined spatial and temporal order. Much of our understanding in these areas has emerged from the studies on the fruit fly, *Drosophila*. To understand the logic behind the process of embryonic differentiation and determination, it is necessary to know how many genes are involved in the embryonic pattern formation, whether each of these genes is unique and what types of pattern alterations are caused by mutations in a single gene. The answer to all these diverse questions lies in the identification and subsequent developmental and molecular characterization of the genes which regulate these developmental steps and cell differentiation. In this review, a recently developed technique, popularly referred to as enhancer trap technique, for identification of *Drosophila* genes has been discussed.

One important aim of developmental biology is to elucidate the complex mechanism controlling early embryonic development. The classical mutagenesis approach to study development is to obtain genetic variants that alter or block developmental decisions during embryogenesis. Several classes of genes have been found to operate during development to establish the final body pattern of *Drosophila*[1-4]. Based on their mode of inheritance, two types of genes have been distinguished – the maternal genes and the zygotic genes. The maternal genes act during oogenesis. The products of maternal genes are provided to the embryo by the germline and the somatic cells of the mother. These maternally derived products function in the synctium to organize the antero-posterior and the dorso-ventral axis of the embryo[5]. Subsequently, the activities of the zygotic genes ensure the origin of diverse spatial pattern in the developing embryo. The zygotic genes act in a hierarchical fashion to divide the embryo into segments and the latter into smaller groups of cells – the compartments. The category of genes classified as homeotic genes specify the individual segmental identity[6-8].

A series of intensive screens for the embryonic lethal mutations leading to pattern defects originally initiated by Nusslein-Volhard and Weischaus[1] have now provided a large list of genes implicated in the genetic control of development in *Drosophila*[1,9-11]. These screens for mutations inducing embryonic lethality have truly been exhaustive and currently it is believed that the technique has come to saturation. At least the prospects of finding new loci by the use of classical mutagenesis screen has receded to the point of unprofitability. Moreover, screens based on mutant phenotype alone may lead to an underestimation of the number of genes required for pattern formation[1-3,9,10]. For example, haploinsufficiency loci, duplicated genes[12], mutations with subtle phenotypes, existence of 'shunts' in the development, and mutations causing developmental arrest before differentiation[2] would have been mostly overlooked in these classical mutagenesis screens. The stage has now been set to search for newer methods of genetic screens for the identification of genes controlling fruit fly development.

One of the possible approaches towards identification of new genes is by virtue of DNA homology. In this reverse genetics approach, for example, the gene *caudal* essential for the normal segmentation process was discovered using the conserved 180 nucleotide sequence as the homeotype[13,14]. Another method involves a screen for gene expression patterns using panels of monoclonal antibodies raised against specific parts or whole of the organism[15]. The antibodies which react with the antigen of the tissue of interest are used to clone the corresponding gene from the cDNA expression library[16]. Screens based on homology, however, have their own limitations. These include the nature of the DNA or the protein probes used and the fact that these depend,

a priori, on functional conservation of the genes in question, whereas there could be many other genes whose identification would nevertheless escape this approach of reverse genetics. In addition, these techniques are time-consuming and genes that are cloned are generally not amenable to immediate genetic analysis.

Enhancer trap technique – a historical perspective

Recently, a powerful and promising technique – the enhancer trap technique – has been developed that permits rapid identification and isolation of new genes regulating fruit fly development[18]. In this technique a P element vector carrying bacterial *lacZ* reporter gene is mobilized to obtain its insertion in a wide variety of genes or their transcriptional enhancers. Subsequently, developmental expression of the *lacZ* reporter gene from its new chromosomal positions is studied to identify novel genetic loci and their functions.

Of the many transposable elements in *Drosophila*, the P elements have attracted maximum attention because several properties of the P elements make them useful for controlled genetic manipulation and fly transformation[17]. Both natural and genetically engineered P element derivatives have been studied extensively. Natural P elements are 2907 bp in length. They have one small and three large open reading frames which appear to encode a single polypeptide with transposase activity and precise inverted repeats of 31 bp that flank the internal sequences[19, 20]. These inverted repeat sequences are essential for P transposition. Transposition of P element is catalysed by the enzyme encoded by its transposase gene. Production of transposase is limited to germline cells due to tissue-specific splicing of the introns of the transposase gene [19–23]. The engineered P elements which are used in the enhancer trap technique or germline transformation of the fruit fly are manipulated to eliminate the transposase gene and replace their internal sequences with markers suitable for their identification following transposition[17]. These P element vectors can now be used for transgenesis of any desired genes (Figure 1). A P vector bereft of its transposase gene, however, is incapable of mobilization on its own. Its mobilization is achieved with the help of transposase enzyme derived from another P element carrying an intact transposase gene[17, 24, 25]. During a typical germline transformation, both vector and helper elements are injected in *Drosophila* polar plasm during early embryogenesis to promote P insertion. Using this strategy, Spradling and Rubin[17] achieved the insertion of *rosy* gene of *Drosophila* in its mutant background to achieve the first landmark of transgenesis in the fruit fly (Figure 1). The subsequent period of research saw an explosion of the literature on fruit fly transformation using a large number of cloned genes.

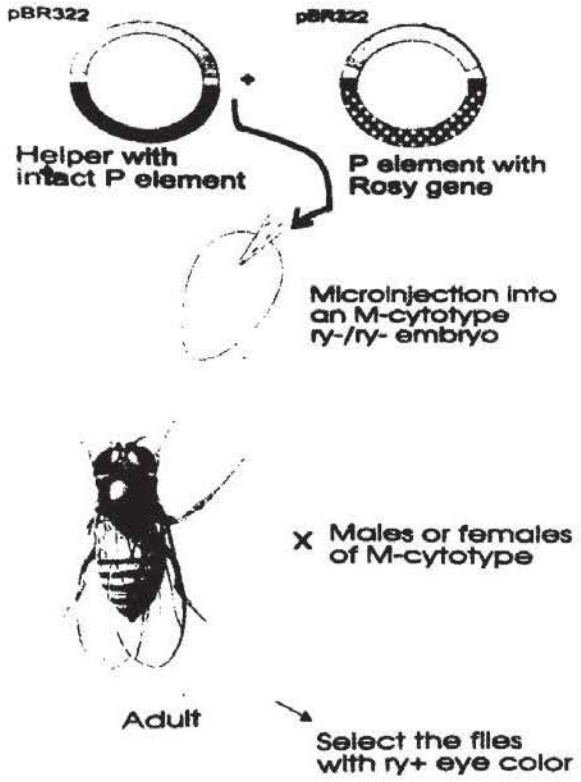

Figure 1. Schematic presentation of the generation of transgenic flies by the microinjection technique. Spradling and Rubin[17] successfully reintroduced the *rosy* (*ry*) gene to achieve fly transformation. The modified P element was constructed by replacing the transposase gene coding region by *rosy* gene and then microinjecting this P element with an intact helper P element into ry^{-}/ry^{-} embryo. The helper element provides the transposase source necessary for transposition of the modified P element. The transformed flies can be detected by the rosy eye colour as the embryo is of M-cytotype, with the adult survivor with rosy eye colour confirming the transformation ry^{-}/ry^{-}.

Development of the enhancer trap technique (Table 1), dealt with in the subsequent sections, owes its origin to a clever manipulation of this technique of fruit fly transformation in the laboratory of Walter Gehring at Basel[18]. This manipulation involved a reconstruction of the transposon P[*lac*, ry^{+}]A having an inframe translational fusion of *lacZ* to the second exon of the P element, the $rosy^{+}$, ry^{+} gene as the adult eye marker, trailer sequences and the rosy polyadenylation site of *Drosophila hsp70* gene on the 3′ end of the *lacZ* gene (Figure 2). This transposon was used for germline transformations to achieve random insertions of bacterial β *galactosidase*, *lacZ* gene[26] at a large number of chromosomal loci. The P *lacZ* vector was microinjected along with the helper (wing clipped) vector[20], which provides the transposase source. Identification of the transformed flies was subsequently made on the basis of the ry^{+} marker in the P *lacZ* vector (Figure 2). The *lacZ* gene in these transformants showed little or no

Table 1. Landmarks in the development and improvization of the enhancer trap technique beginning with fly transgenesis

Landmarks	Technique	Transposon designed	Helper used	Reference
Transgenesis	Microinjection	P element harbouring the *ry* gene of P element *Drosophila*	Intact	17
Transgenesis to detect enhancer	Microinjection	P (*lac*, ry^+) A vector harbouring *lacZ* reporter gene	p π25.7 *wc* (wing clipped) P element	18
Generation of stock with stable transposase source from an integrated P element	Microinjection	*ry* Δ2–3 element		25, 28
P-element-mediated insertional mutagenesis	Transposon mobilized by genetic cross	*pUChsneo* vector	hsp70 promoter fused to *white* and *transposase* gene inducible transposase source	27
Generation of transposant lines/tissue-specific markers by enhancer detection/ trap technique to study *Drosophila* development	Same as above	P *LArB* or P *lacW* vectors	Stable Δ2–3 element	30–33
Modified P element constructed by promoter fusion of genes of interest to *lacZ* reporter gene to promote preferential insertions	Same as above	P *en lacZ* P *LArB* P *eve lacZ* P *ftz lacZ* vectors	Same as above	35, 36, 39
Construction of *hobo lacZ* element, followed by generation of. a. Transformant lines b Transposant lines	a Microinjection b. Transposon mobilized by genetic cross	H(pHLw2) with *miniwhite* and *lacZ* reporter gene	P(ry^+, HBL1)	57
Site-directed expression of the gene using the yeast transcriptional activator Gal4	Transposon mobilized by genetic crosses	pGATB pGATN pGawB pUAST vectors	Stable Δ2–3 element	60

The origin of enhancer trap technique can be traced from fly transformants[1] with subsequent innovations. Transgenic flies are those which receive a gene by P-mediated germline transformants, whereas transposant flies[32] are those which receive the P transposon through germline mobilization of the element in the parental flies in a genetic cross.

endogenous activity but remained responsive to the developmental regulation of the enhancer elements of the genes in the neighbourhood. Transformation of the fruit fly with this element revealed a strikingly large number of genetic loci which could developmentally regulate the expression of the *lacZ* reporter gene. This technique opened an immense prospect of search for new genes based on developmental expression of the *lacZ* reporter gene. It thus provided a means of first determining a tissue- and developmental-stage-specific expression of a given gene and its subsequent genetic and molecular characterization[18]. The technique, however, suffered from the limitation of the arduous task of microinjection in thousands of embryos and identification of transformed lines before one could search for developmental expression of the *lacZ* reporter gene. The very magnitude of the physical labour involved in this kind of approach appeared to be a major hurdle in large-scale application of this technique. Two independent groups of workers, one at the laboratory of Allan Spradling at Carnegie Institute, Washington[27], and the other at the laboratory of William Engels at University of Wisconsin[28], achieved integration of a stable source of transposase gene, which eventually circumvented the problem of large-scale microinjection. Cooley *et al.*[27] transformed flies with P element where the transposase gene was placed under the regulation of *Drosophila* heat shock promoter (*hsp70*) that permitted its activation under heat shock conditions (Figure 3).

In a genetic cross this integrated helper element with an inducible source of transposase could catalyse the transposition of recombinant P element to new genetic locations. The mobile P vector carried the necessary markers (Figure 3), which made the identification and molecular cloning of the genomic fragments adjoining the insert almost instantaneous[27]. The large number of inserts in the Spradling laboratory nearly generated a library of stocks with P insertions at various genetic loci.

The stable source of transposase generated in the Engels Laboratory[28] marked a further improvement over the inducible transposase source devised in the Spradling laboratory on two accounts – firstly, the stability of the transposase source was extremely high and, secondly,

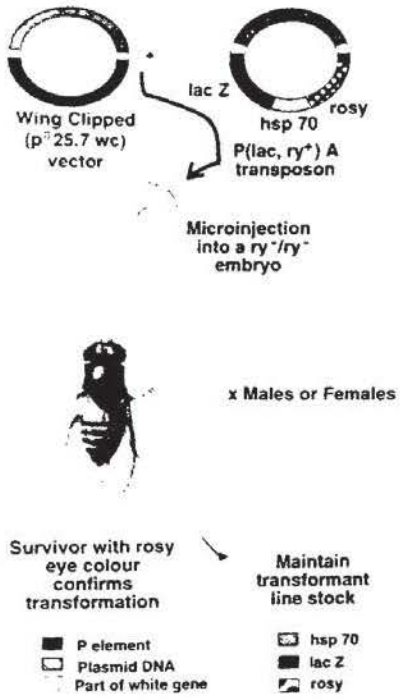

Figure 2. Graphic presentation of the technique of the first case of the enhancer trap employed. O'Kane and Gehring[18] designed the P (*lac*, *ry*⁺)A transposon harbouring the *lacZ* and *rosy* reporter gene. The modified P element was microinjected along with the P 25.7 *wc* (wing clipped) vector (where the flanking inverted repeats of the P element are clipped off which are necessary for its insertion and hence acts as a stable transposase source) into the *ry*⁻/*ry*⁻ embryo. The transformant survivor adults are recognized on the basis of rosy eye colour. The reporter *lacZ* gene driven by a weak P promoter expresses under the regulation of the genomic enhancers of the *Drosophila* genome, which were subsequently detected by histochemical staining for β-galactosidase.

the transposase was coded constitutively in germline and somatic cells without the need for a heat shock. This element, called the Δ2–3 element[25, 28], lacked the second and third introns of the transposase gene. In the absence of these two introns the transposase activity appeared to become ubiquitous and not restricted to the germline alone[25]. While working with this P element vector with modified transposase gene, Laski *et al.*[25] obtained an insertion of the element on chromosomal position 99B7–10 of the polytene chromosome map near the tip of the right arm of the third chromosome. Robertson *et al.*[28] characterized this element and found somewhat fortuitous loss of the ability of this element to transpose to new genetic locations. This stable and constitutive source of P transposon brought a sea change in the subsequent course of investigations, leading to a great simplification of the enhancer trap techniques (Table 1).

The enhancer trap technique which is currently being practised involves mobilization of an engineered element akin to the one devised by O'Kane and Gehring[18] in simple genetic crosses with the help of a stable endogenous source of transposase of the Δ2–3 (99B7–10) line. This completely did away with the need for microinjection to obtain chromosomal insertions of P *lacZ* vectors (Figure 4 and Table 1). Once liberated from the constraints of microinjections, it was inevitable that the mobilization of the P *lacZ* vector was carried out extensively by several independent groups of investigators that led to an explosion of information about developmental expression of a wide range of *Drosophila* genes and identification of novel genetic loci[29–34]. In this somewhat oblique version of reverse genetics, search for a given gene was now entirely based on developmental expression of the *lacZ* reporter gene, followed by its genetic and molecular characterization.

Two groups of investigators contributed immensely to this new era of developmental genetics, one in the Walter Gehring laboratory at Basel[30–32] and the other at Y. N. Jan laboratory at San Francisco[33]. Both these screens employed similar strategies that involved mobilization of the P *lacZ* vector in genetic crosses under the influence of a stable source of transposase[28]. The P *lacZ* vector used in these two cases differed primarily with respect to the markers used for identification of flies carrying new transpositions (also called transposant flies[32]). Both these screens were immensely successful in identifying a large number of new genetic loci involved in a number of developmental pathways that include (1) embryonic segmentation[32, 33, 35–37], (2) neurogenesis[30, 33, 38, 39], (3) homeosis[22, 29, 40], (4) oogenesis[29, 31] and (5) spermatogenesis[41]. The list is long and ever-increasing. It appears that it would be several years before the drosophilists achieve a complete characterization of these new-found genes.

Meaning and utility of *lacZ* reporter expression

In P-element-mediated enhancer detection or enhancer trap technique, it is presumed that the expression of *lacZ* reporter gene is influenced by regulatory elements called the enhancers. These elements have the remarkable property of operating over a distance of up to several kilobases regardless of their orientation in DNA. Enhancers promote the stage-specific expression of genes under their regulation[32]. These sequences were first discovered in the deletion mutants of the SV40 virus preceding the cap site for the SV40 early transcription unit and were found to enhance the expression of the viral gene[42, 43]. While it is likely that developmental expression of the *lacZ* reporter gene in fruit fly is also activated by such enhancer elements, it is also obvious that in a larger number of cases the P *lacZ* vector insertion takes place within the transcription unit of a structural gene (for example, in *teashirt*, *tsh* gene[34]). In this situation one cannot be certain about the relative influence of a distant enhancer element versus the

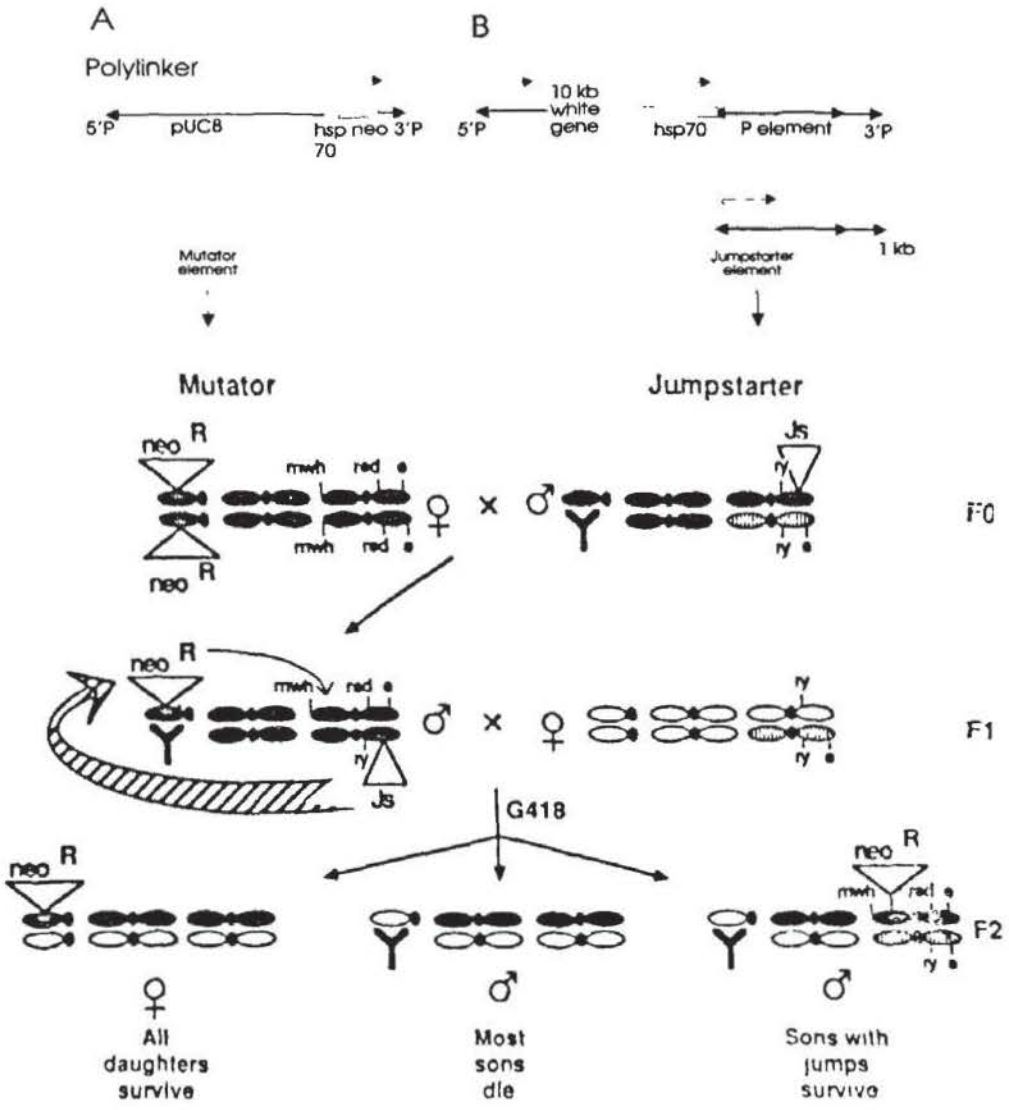

Figure 3. P-element-mediated insertional mutagenesis technique of Cooley *et al.*[27], where the P element is manipulated by introducing *neo*^{R} (neomycin resistance) gene into it and the transformant lines are generated. A P transformant line with P element insertion on 9C position of the X chromosome with unmarked second chromosome and *mwh red & e* on the third chromosome was used for mobilization of the P element in a genetic cross. The transposase source was provided by a designed jumpstarter element in the other strain. The jumpstarter contains a single autonomous P element (JS) at position 90A on a *ry*^{506} third chromosome and a *TM3*, *ry*^{RK}, *Sb e* balancer chromosome (hatched). F_1 sons containing P elements were recovered from the crosses with the aid of markers and mated individually to virgin females containing the *TM3*, *ry*^{RK}, *Sb e* and *ry*^{506} third chromosome. Larvae of this cross were subjected to heat shock at 37°C for 30 min for 3 to 4 days after the cross which will initiate the mobilization of the P element. The progeny bearing the P insertion was screened on a neomycin medium (G418 medium). (Courtesy Cooley *et al*[27])

regulatory elements within the endogenous gene. In brief, therefore, the popular reference to the technique as enhancer trap is more in the nature of corollary to the prokaryotic terminology.

The activity of the enzyme β-galactosidase encoded by *lacZ* can be detected cytochemically by its action over the chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), which is then oxidized to form a blue dye 5,5′-dibromo-4,4-dichloro-indigo. The most striking aspect of the enhancer trap technique is the ease and the reasonably high fidelity with which the *lacZ* reporter gene reflects the pattern of expression of the endogenous genes. The most crucial evidence in this regard has been the demonstration that insertions at a known genetic location display the expression pattern of the *lacZ* reporter gene akin to that of the endogenous genes (Figure 5). The insertions at *wingless* (*wg*) and *engrailed* (*en*) loci illustrate these points. Insertion of the *lacZ* vector at the *en* or *wg* loci[35,36] reveals both embryonic and imaginal disc expression characteristic of the zones of RNA/protein expression of the respective genes (Figure 5).

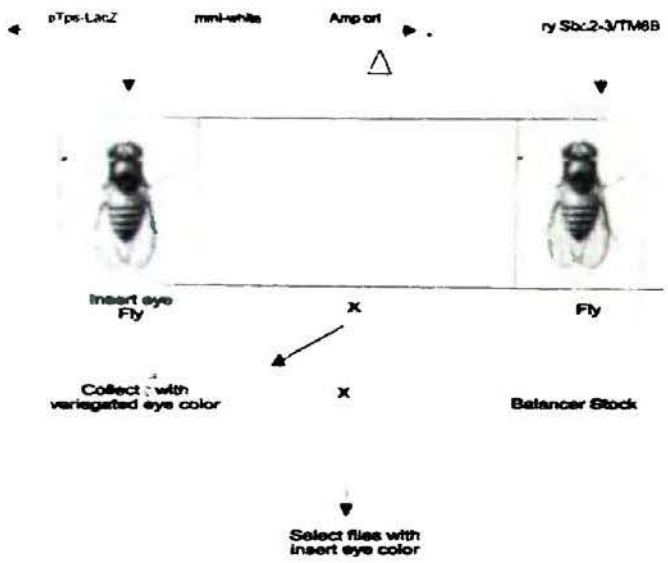

Figure 4. Scheme of mobilization of P *lacW* transposon to generate the enhancer trap lines by genetic crosses. Flies harbouring the modified P element having *lacZ* and *mini white* reporter genes were crossed to the Δ2–3 element stock, which provides the transposase source to mobilize the modified P element, and the transposant lines were generated by mating the transposant males to suitable balancer stocks. (For details see ref. 33.)

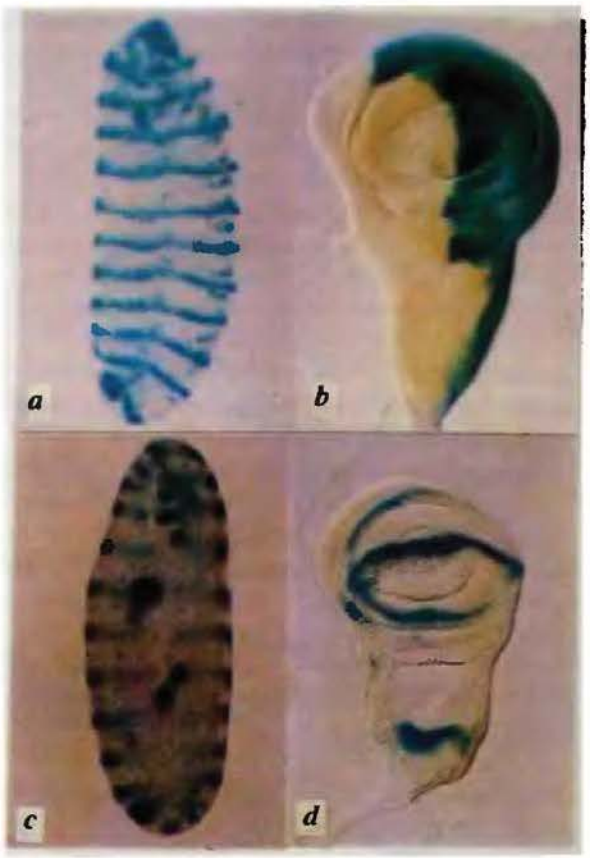

Figure 5. Reporter *lacZ* expression bears correspondence to spatiotemporal expression of the gene: *a, b*, *lacZ* expression detected in the embryo and imaginal disc of the transposant line with P element insertion at the *en* locus; *c, d*, *lacZ* expression in embryo and imaginal disc in the *wg lacZ* insert line. (These stocks were kindly provided by Judith Kassis[35, 36] and are available in Drosophila Stock Centre, DAVV, Indore.)

A major advantage of this technique is its ability to detect genes which do not give an obvious morphological defect when mutated but are required for normal development. The gene *elav* is an example of this category of genes, which plays an important role in neurogenesis[44, 45]. The embryonic phenotypes of *elav* mutants are so subtle that these would have been missed by the screens using anti-HRP labelling of the nervous system. The enhancer trap technique, on the other hand, helps in detecting the spatiotemporal expression of such genes by tagging the reporter gene to these genes. The reporter gene expresses under the regulation of the enhancer elements of the gene. Consequently, the reporter gene expression corresponds to the spatiotemporal expression of the gene (Figure 5). This technique also helps in identifying the genes that play an important role at multiple developmental stages. Such genes would be difficult to identify in the classical mutational screen as their functional requirements during later stages of development would be obscured by earlier defects.

One can also exploit the *lacZ* reporter gene expression of a given genetic locus to study its regulatory hierarchy in a suitable genetic background. This is generally achieved by placing the *lacZ* enhancer trap insertion in a background of mutant genes which are likely to be involved in its developmental regulation. For example, repression of *cubitus interruptus* (*ci*)[37] in the posterior compartment by *en* was discerned by studying its *lacZ* reporter expression in an *en* mutant background. This provides a greatly simplified approach to study the complexity of interaction of the genes regulating *Drosophila* development and ascertaining their hierarchy of action. The enhancer trap technique also provides a simultaneous means of P mutagenesis and generating molecular tag on the desired gene. Insertion of the P *lacZ* vectors may cause inactivation of the endogenous gene that results in its lethal mutations, while its *lacZ* reporter gene simultaneously provides the developmental expression pattern in the mutant heterozygotes. Thus, one can have the twin advantage of learning about the expression of a given gene and simultaneously looking into its mutant phenotype. From a given P insertion, lethal or viable, one can further mobilize the P element in genetic crosses[32, 33]. This leads to the excision of the P element which is generally imprecise. During these imprecise excisions, P element can cause deletions around the site of insertion, thereby generating a large number of mutant alleles of the desired genetic loci. In effect, once a P insertion on a desired locus is obtained, one can follow its developmental expression and study its

mutant phenotype by excising the P element within the course of a few months. This means a breathtaking speed of investigations, which otherwise would have involved years of painstaking work.

The technique also helps in cloning and characterization of the *Drosophila* genes. The engineered P *lacZ* vector inserted contains a rescuable plasmid[32, 33] which makes the isolation of the molecular clone of the genetic loci of insertion easy (Figure 4). Beginning with plasmid rescue using suitable restriction enzymes, the genomic clone adjoining the P insert can be isolated and this can be followed by a chromosome walk to isolate the overlapping DNA fragments from the *Drosophila* library[32]. These genomic fragments can be characterized and used to identify the transcripts in northern blots to initiate the subsequent course of molecular characterization of the locus.

Other applications of the enhancer trap technique

The enhancer trap technique in combination with methods for mobilizing single P elements[27, 28] has allowed the production of numerous tissue-specific patterns of β-galactosidase expression that can be used to study cell lineage relationships in the growing embryos, imaginal discs or other internal organs[30, 33, 39]. The great variety of *lacZ* patterns found in different lines can be used as cell type or position-dependent markers to study *Drosophila* development and cell lineages[39]. A large number of cell types of *Drosophila* peripheral nervous system (PNS) have now been identified by this technique. This permits independent labelling of virtually every cell type in the PNS, e.g. lines staining type II neurons (multiple dendrite neurons), subsets of chordotonal stretch, receptor cells, and subsets of external sensory organs[33, 39]. Similarly, enhancer trap lines marking the various components of the central nervous system (CNS)[46] and other organs like mid-line cells[47] have been generated.

Drawbacks of the enhancer trap technique

Correspondence of spatial and temporal pattern of the *lacZ* reporter gene expression with that of the endogenous gene is crucial in the interpretation of the results. In this regard perdurance[48], meaning a longer persistence of the gene product while the gene itself has shut off, poses several problems. Perdurance of β-galactosidase activity may give an erroneous impression of a longer duration of activity of the endogenous gene. Perdurance may also obscure the identification of genes with a dynamic pattern of expression, such as the *string* gene, which is transiently expressed during the 13th nuclear division of embryonic cleavage[49].

It is also likely that in an enhancer trap screen one may easily miss or overlook the genes that are expressed ubiquitously but play important developmental roles in specific regions or cell types. For example, the gene *Notch, N* plays an important role in allowing cells in the ventrolateral region of the embryo to make a choice between assuming epidermal or neuronal fate[50, 51]. Developmental expression of *N*, however, takes place in the dorsal as well as ventral regions during embryogenesis[52]. A comparable expression of *N*-specific *lacZ* reporter gene in an enhancer trap screen would obscure its developmental role.

Finally, it is important to state that an interesting expression pattern may not always be indicative of the true domains of genetic function of the endogenous gene. For instance, although many other photoreceptor cells in addition to R7 photoreceptor express *sevenless* even after their determination[53, 54], mosaic studies have shown that *sevenless* function is required only in the R7 photoreceptor cells[50, 54, 55]. Interpretation of the *lacZ* reporter expression, therefore, demands recognition of these aspects and in addition may demand support from subsequent *in situ* RNA or protein localization.

Perspectives

The advantages and utilities of the technique outweigh some of its disadvantages. The popularity of the technique is further enhanced by the various modifications which provide increased versatility. One of the promising aspects is to engineer a P *lacZ* vector to alter its specificity of insertion. In a study to analyse the regulatory region of *en*, P *lacZ* vectors carrying different fragments of the *en* promoter were used to transform flies. Interestingly, 7 out of 20 insertions were obtained at or near the *en* locus, which indicated that the regions upstream of the *en* structural gene confer the P *en–lacZ* vector a site preference to the endogenous *en* locus in a pairing-dependent fashion[56]. Taking cue from these observations Kassis *et al.*[36] and Perrimon *et al.*[39] transformed flies with P *lacZ* vectors carrying different fragments of the *en* promoter region. These transformed flies were subsequently mated to flies with a stable source of transposase to mobilize the element in a typical enhancer detection scheme[31–33, 35, 36, 39] (Table 1, Figure 4). The modified *lacZ* vector showed preferential insertion of the element to genes which express in stripes during embryogenesis[35, 36] and to those genes which appear to be expressed during neurogenesis, segmentation and pattern formation[39]. These results show that P insertion, which is generally believed to be random, can be endowed with site preference with suitable modifications (Table 1).

Another modification of the original P element enhancer trap technique which holds immense potential is the *hobo* enhancer trap technique[57], which employs

hobo lacZ vector instead of P *lacZ* vector. *hobo* elements resemble P elements in having inverted terminal repeats and transpose via DNA intermediates[58]. However, despite similarities, they have certain differences which lead to their varying specificities for insertion. Identification of a large number of *hobo lacZ* enhancer trap lines on the second chromosome revealed an altered insertional specificity of the element[57]. This *hobo lacZ* vector thus holds promise for identification of the loci which escaped detection using P *lacZ* vectors.

The Gal4 enhancer trap technique, also called the second-generation enhancer trap, is a modified version of the original enhancer trap technique. This technique is employed for the study of site-directed expression of the *Drosophila* genes (Table 1). The technique makes use of the yeast transcription factor Gal4, which has no target sites in *Drosophila* genome but can be made to express itself when provided with a Gal4 responsive promoter, UAS_G (ref. 59, 60). In this technique a P element harbouring the *Gal4* gene is constructed and flies are transformed by microinjection technique[18]. The P element bearing the *Gal4* gene is mobilized in genetic crosses using the jumpstarter strain, Δ2–3 element which provides a stable transposase source[28] (Table 1, Figure 6). New insertions in *Drosophila* genome are random and bring Gal4 expression under the control of a diverse array of genomic enhancers. An important feature of this technique is that a conditionality is involved in which the expression of a gene depends on the presence of both Gal4 and its responsive promoter. UAS_G, which are maintained in separate stocks. The gene expression is, therefore, detected only in the progeny where both Gal4 and its promoter UAS_G are present. In the UAS_G stock the promoter is fused to β *galactosidase* gene, whose transcription, therefore, is driven by Gal4-binding sites[59,60]. Brand *et al.*[60] generated strains in which *lacZ* expresses under *Gal4* gene, which in turn is under the regulation of the enhancers of the *Drosophila* genes.

This technique can be employed to study the ectopic expression of genes by generating stocks by fusion of the gene of interest with the UAS_G promoter. The flies of this stock are crossed to the flies of stocks where Gal4 is under the regulatory elements of the gene of interest with known tissue-specific expression. For example, ectopic expression of the homeotic gene *Abdominal* in the domain of *twist* gene was achieved using this technique[61]. Ectopic gene expression can be studied to ask important questions of function of the genes in development. One can foresee a wide range of applications of this technique to achieve misexpression, to make fruit fly developmental biology far more interesting and exciting in the immediate future.

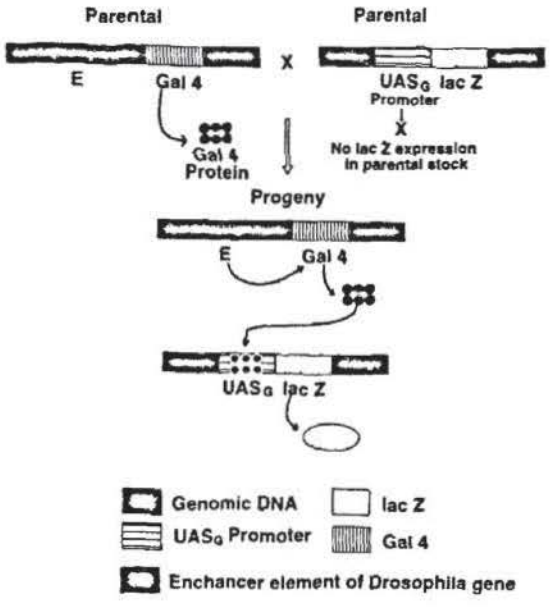

Figure 6. Schematic diagram of the Gal4 enhancer trap technique to present the site-directed expression of the gene. Stocks bearing the UAS_G promoter fused to the *lacZ* gene and strains with Gal4 insertion in the *Drosophila* chromosome were generated by the enhancer trap technique[18]. The Gal4 expresses only after binding to the UAS_G promoter present in the other stock. Gal4 shows spatiotemporal expression under regulation of the enhancer element of the gene of *Drosophila* near which it inserts. The progeny from the cross of the flies of the two stocks presents the *lacZ* expression mediated by Gal4 expression, which is under genomic enhancers of the *Drosophila* gene where the P element bearing the *GAl4* gene got inserted. (Adapted from Kaiser[61].)

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