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A screen for genes expressed in *Drosophila* imaginal discs

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ABSTRACT The development of *Drosophila* imaginal discs serves as a model system to understand how genes determine the shape and size of an organ. The identification of genes involved in this process is an important step towards this goal. Here we describe a P-element based enhancer trap screen for genes expressed in the larval imaginal discs. Our aim was to establish a large collection of enhancer trap lines each showing expression of Gal4 in imaginal discs. To this end, we improved the well established P-element vector pGawB in order to obtain higher *in vivo* transposition frequencies. In addition we chose an F1-screening approach using UAS-GFP as a reporter gene. This system permits the efficient screening of larval and pupal stages of living animals and the detection of imaginal gene expression patterns through the transparent cuticle. The procedure has been optimized for high-throughput. 2'000 P-element insertions have been established which exhibit expression in imaginal discs.

KEY WORDS: *imaginal disc, enhancer trap screen, Gal4, P-element, UAS-GFP*

Introduction

The limbs of the adult *Drosophila* develop from imaginal discs. These discs invaginate from the embryonic ectoderm as simple pouches of epithelium. During larval development the growing disc epithelia are progressively subdivided into domains of different gene activities. By the end of the 3rd larval instar the disc epithelia have almost reached their final size, and patterning is largely complete. At metamorphosis this comprehensive spatial information is translated into a series of cellular and anatomical changes to produce an appendage, such as a leg or a wing.

Identification of the whole spectrum of differential gene activity within the disc epithelium would represent a major step towards elucidating the developmental route leading from genes to structures. The enhancer trap approach (O'Kane and Gehring, 1987; Bellen *et al.*, 1989; Bier *et al.*, 1989; Wilson *et al.*, 1989) has been a breakthrough for the identification of new Drosophila genes on the basis of their spatial and temporal expression pattern. Many developmentally important genes have been identified by this method. With the aim to upscale such screens we sought to circumvent some of the time-limiting aspects of the enhancer trap method. In most previous screens individual lines are established randomly, each carrying a new insertion of an enhancer trap P element. In a second step each of these lines is individually analyzed for the expression pattern of the enhancer trap reporter gene. However, for the study of a single specific developing organ, such as the wing imaginal disc, the majority of the established insertions may show no reporter gene expression in the tissue of interest (Bier *et al.*, 1989; Brand and Perrimon, 1993). To overcome this problem and to identify a large number of genes involved in patterning of the wing disc, we employed the Gal4 enhancer trap method (Brand and Perrimon, 1993) in a manner that enabled a one-step screen directly in living 3rd instar larvae. Of ca. 40'000 new Gal4 insertions screened, 2000 lines were established, all of which are expressed in the wing disc. 75% of these exhibit a restricted pattern of expression. Apart from many known expression patterns numerous novel patterns of gene expression were identified.

Results and Discussion

General Limitations of P Element-Based Enhancer-Trap Screens

Generation of Large Numbers of Independent P-Insertions. To obtain new enhancer-trap insertions flies carrying a starter P element are crossed *en masse* with flies carrying a stable source of transposase. In somatic as well as germ cells of the resulting progeny (referred to as 'jumpstarters'), the P element can be excised and reinserted into new genomic locations. Because of pre-meiotic transposition events, which can lead to identical insertions, only one resulting transposant from each jumpstarter cross is used to establish a new line. Ideally therefore, jumpstarter flies are crossed individually, and the number of crosses set up will determine the maximal number of transposants recovered. If the transposition frequency of the starter element used is low, not every cross with a single jumpstarter will yield a new insertion. For these two reasons the generation of substantial numbers of transposants is limited and involves at least the same number of crosses.

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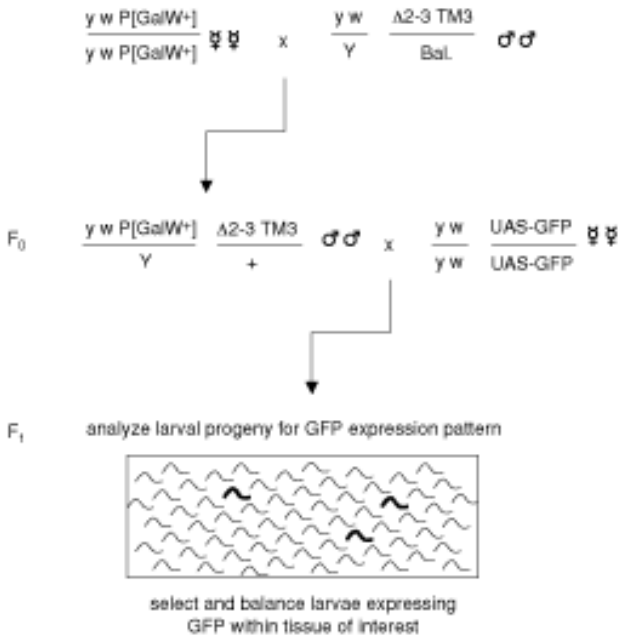

Fig. 1. Genetic scheme for generating and screening wing disc specific enhancer trap lines. *For details, see text.*

Analysis of the Enhancer-Trap Expression Patterns. Approximately 35% of new insertions do not express the UAS reporter gene due to inactivation of the Gal4 gene during transposition (Bellen *et al.*, 1989; Bier *et al.*, 1989; Brand and Perrimon, 1993; Calleja *et al.*, 1996). Of those lines that have an intact Gal4 element, the majority fall into three non-relevant categories (Bier *et al.*, 1989): no expression at all, ubiquitous expression, or no expression in the tissue of interest.

The progeny of each transposant has to be analyzed for the expression pattern caused by its enhancer trap insertion. In most Gal4-based screens, a UAS-lacZ transgene was used as a reporter. Thus many rounds of dissections are required to determine the imaginal disc expression pattern of all lines. A notable exception was the screen carried out by Calleja *et al.* (1996) who used a *UAS-yellow* reporter to visualize Gal4 expression in adult, living individuals. A theoretical limitation of the *UAS-yellow* approach, however, is the potential existence of dynamically changing expression patterns in imaginal discs which might not be faithfully represented in adult appendages.

A GFP-Based F1 Enhancer Trap Screen

Here we tried to avoid some of the above mentioned limitations of enhancer trap screens by using an F1 set up in conjunction with a GFP reporter (Fig. 1). Following a mobilization event of a Gal4-containing transposon (see below), the Gal4 expression pattern driven by endogenous enhancer elements can be visualized by a *UAS-GFP* transgene. Since the *Drosophila* larva is semi-transparent, a large scale F1 screen of specific GFP expression patterns in wandering 3rd instar larvae is possible. Larvae with specific wing imaginal disc expression can be detected under a UV stereomicroscope, collected and propagated. The scheme has the following advantages: (1) Jumpstarter males can be crossed *en masse* with *UAS-GFP* homozygous females, as no lines have to be set up before screening for novel expression patterns. (2) Progeny larvae carrying new insertions can be identified based on the Gal4 activity revealed by GFP expression, and not according to other markers associated with the P element. (3) Lines only need to be established from individuals that show specific expression in the wing imaginal disc, thus circumventing the need to establish many non-relevant lines. (4) The Gal4 expression pattern is only analyzed by dissection in cases known to exhibit interesting disc expression.

Improvements to Enhance the Efficiency of the F1 Screen

To obtain a maximal number of new transposants showing wing imaginal disc expression with a minimal amount of resources, various measures were taken on several levels.

(1) The first limitation we aimed to bypass concerned the mobilization frequency of the Gal4 enhancer trap P element. For unknown reasons the pGawB element of Brand and Perrimon (1993) has a mobilization frequency which is an order of magnitude lower than that of the placW element of Bier *et al.* (1989). We constructed a hybrid element, PGalW, based on PlacW retaining the high mobilization frequency yet expressing the Gal4 reporter of pGawB instead of lacZ. Average transposition frequencies of this new P element were higher than 10% (see Materials and Methods for how this rate was assessed).

(2) We then screened our potential starter P lines for insertions that do not show any imaginal disc expression. Hence any GFP signal appearing in disc tissue of the progeny must stem from a new integration event. We noticed that virtually all pGawB insertions show Gal4 expression in salivary gland cells, regardless of their integration site. The same was the case with our PGalW element. These constructs must carry a fortuitous salivary gland enhancer element. The expression in salivary glands slightly complicates the visual screening of wing imaginal disc patterns, as both tissues are in close proximity. We were able to map this enhancer to the 5' UTR of the Gal4 mRNA (derived from sequences of the *hsp70* gene). When these leader sequences were removed, expression in salivary gland cells was abolished (data not shown). However, such derivatives of

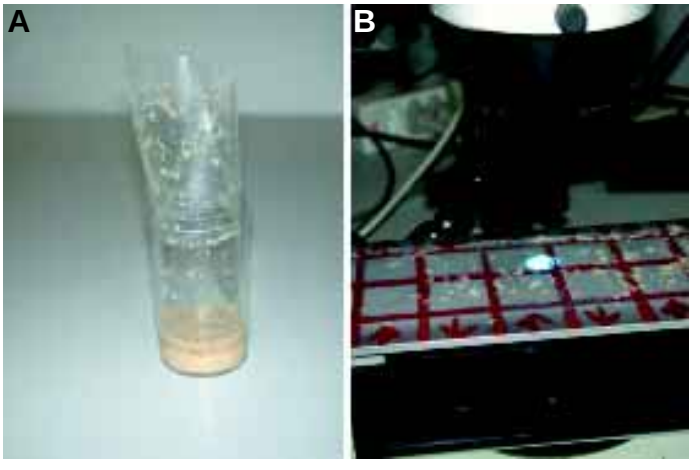

Fig. 2. Equipment used to facilitate the screen. (A) *A transparency covered with 3rd larval instar progeny resulting from a cross between jumpstarter males and UAS-GFP females.* **(B)** *A populated transparency placed onto a cooling device under a UV stereomicroscope.*

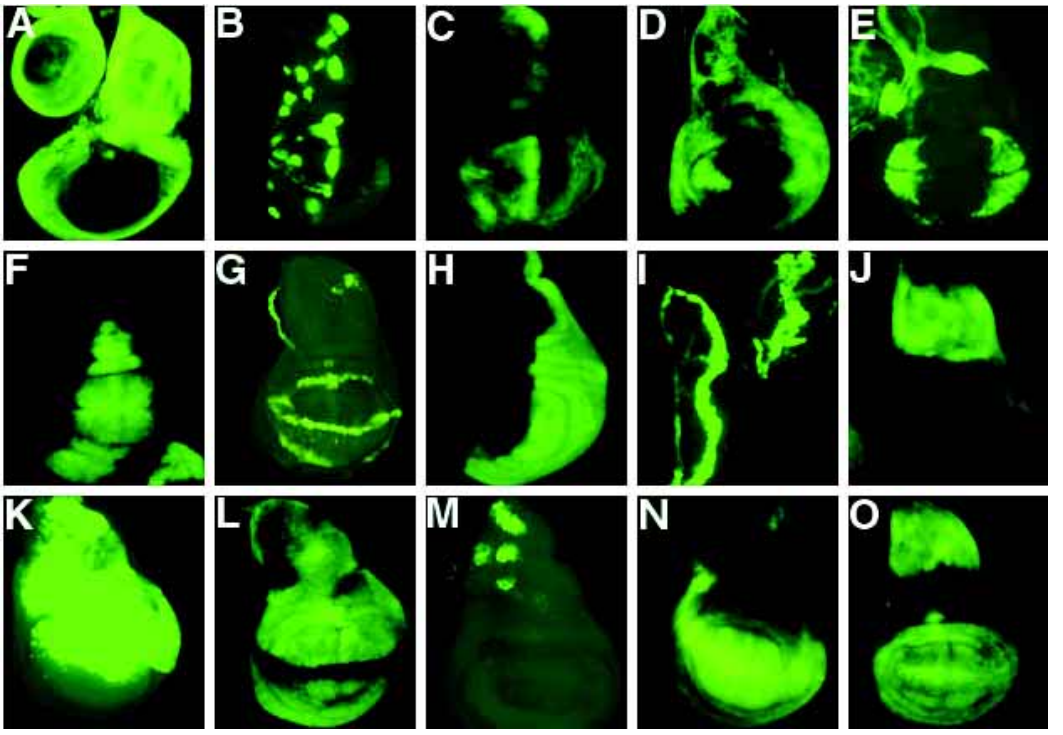

Fig. 3. Gal4 expression patterns in wing imaginal discs of newly isolated enhancer trap lines. (A) teashirt (tsh), **(B)** scabrous (sca), **(C)** mtv, **(D)** brinker (brk), **(E)** nd23, **(F)** optomoter-blind (omb), **(G)** wingless (wg), **(H)** hedgehog (hh), **(I)** patched (ptc), **(J)** nd637, **(K)** apterous (ap), **(L)** fringe (fng), **(M)** nd559, **(N)** scalloped (sd), **(O)** D-frizzled-3 (fz3).

PGalW exhibited reduced functionality as enhancer trap vectors and were therefore not used in our screen.

(3) We also aimed to optimize the sensitivity of the UAS-GFP reporter system. Multiple new UAS-GFP transgene insertions were established, and the two most sensitive of those which showed homozygous viability were selected and recombined onto a single third chromosome (yielding line 403.9+14). To distinguish the Gal4 element (marked with *white*$^{+}$) and the UAS-GFP transgene, we used a *yellow*$^{+}$ minigene as a marker for the latter.

(4) There is an inverse correlation between the survival rates of the screened larvae and the time they are exposed to UV light. Hence, the inspection time in the screen needs to be kept to a minimum (< 30 seconds). Wandering 3rd instar larvae increase their movement while trying to avoid the UV beam. This renders the analysis of the GFP fluorescence under time constraints very difficult. By cooling the larvae during the screen their movement can be reduced and their survival rate increased.

(5) There is a technical difficulty of collecting thousands of larvae and placing them under the UV microscope. The jumpstarter males were crossed to UAS-GFP females in bottles and we used a clear plastic sheet (from overhead transparencies) to line the inner surface of the bottles (see Fig. 2A). When the transparency was covered by wandering 3rd larval instar progeny, it was replaced by a new one. For screening the transparency was then placed onto a cooling device under a UV stereomicroscope with its populated side up (Fig. 2B).

Numbers

A total of 2'000 bottles were set up, each containing 15 jumpstarter males *(PGalW; Δ2-3 TM3 Sb)* crossed to 70 homozygous *UAS-GFP* virgins. Three to four transparencies were screened per bottle, thus a total of ca. 7'000 transparencies were screened. On average each transparency contained 150 3rd instar larvae, of which 50% contained the heterozygous PGalW element (75 per transparency). In total, over 400'000 larvae containing the PGalW element were screened under the UV microscope.

To avoid a potentially biased target spectrum of a particular starter element, several starter insertion sites were used for the screen. The transposition frequency determined for all these lines (based on segregation of the P element-associated *white*$^{+}$ marker) was found to be higher than 10%. Based on the number of larvae screened (400'000 containing the PGalW element) and the transposition frequency of the starter elements we estimate that ca. 40'000 new P-insertions were generated. In this screen 2'000 wing disc insertions were recovered into stable lines; 75% of those exhibited a restricted pattern of expression and 25% showed ubiquitous expression within the wing disc.

About 15% of the established lines contained more than a single P element (in some cases the initial starter P element was still present). The incidence of more than a single insertion per line presents a major problem for the identification of the gene responsible for the Gal4 expression pattern. For many interesting lines, therefore, we carried out *in situ* hybridization to polytene chromosomes. About 10% of the insertions were *white*$^{-}$. Those insertions required a more complicated balancing scheme, as the Gal4 element has to be followed in the presence of a *UAS-GFP* transgene at larval stages throughout all crosses by UV microscopy. Based on sampling a few transparencies, we estimate that about 10 to 15% of all new insertions are expressed in the wing disc in a specific manner. One would expect therefore to recover about 4'000 new insertions exhibiting wing disc expression. The fact that only about 2'000 lines were established – representing about 50% of the expected number – can be attributed to the reduced viability caused by the UV screening (survival rate is ca. 70%) and to the failures to establish lines from individuals which also inherited the transposase chromosome and whose P element is hence unstable.

Yield of the Screen

The lines were categorized according to their expression pattern. Many of the familiar expression patterns corresponded to insertions in the respective known genes. We recovered Gal4 insertions in wing patterning genes, such as *hedgehog*, *patched*, *wingless*, *apterous*, *brinker*, *optomoter-blind*, *frizzled-3*, *fringe*, *scalloped*, and many others (for examples, see Fig. 3). Other insertions with interesting expression patterns are currently being subjected to a molecular analysis. The P element PGalW permits 'plasmid rescue'-cloning of adjacent genomic sequences. Together with the full sequence of the Drosophila genome it is therefore possible to efficiently determine the precise site of each P element insertion.

Conclusions

The screen we performed is based on a combination of the enhancer trap system (Bellen *et al.*, 1989; Bier *et al.*, 1989), the Gal4 method (Brand and Perrimon, 1993), and the approach of Calleja *et al.* (1996) to screen living animals. Our system allows one to efficiently screen larval and pupal stages of living animals and detect gene expression patterns through the transparent cuticle. The key improvements to previous enhancer trap screens for larval expression patterns are the use of primary transposition events (eliminating the time-consuming effort to establish individual lines first) and the detection of gene expression patterns *in situ*. The procedure has been optimized for high-throughput and allowed the establishment of ca. 2'000 lines with reporter gene expression in wing imaginal discs. This collection should lead to the identification of many novel genes involved in wing development and therefore serves as a powerful resource for better understanding the genetics underlying pattern formation.

Materials and Methods

Frequency of Transposition

The mobilization frequencies for the different PGalW starter lines were determined by analyzing 20 individual crosses, each consisting of one jumpstarter male, carrying both the initial PGalW element and the transposase transgene (Δ2-3), mated to *y w* virgins.

For X-linked P elements the mobilization frequency was defined as the ratio of red-eyed w^+ progeny males to total number of progeny males. For insertions on autosomes the PGalW element in the jumpstarter males was placed over a marked balancer chromosome. The proportion between red-eyed w^+ progeny, which carry the marked balancer chromosome to total number of progeny that carry this balancer, gave the mobilization frequency. The highest transposition frequencies for the starter P element lines were between 10 and 18%. The mobilization frequency obtained in this way overestimates the true number of transposition events due to pre-meiotic transpositions that give rise to sibling transposants carrying the same insertion. Taking this into account we calculated the total number of new insertions generated in our screen based on an approximate transposition frequency of 10%.

The individual crosses of jumpstart males were also used to estimate the total number of new insertions generated in the screen in a different way. The proportion of matings in which transposition had occurred was found to be 100%. 2'000 crosses were set up in the screen, each with 15 jumpstart males. In total, 30'000 jumpstart males were mated which should give rise to a minimum of 30'000 transposants.

Fly Stocks

Four starter PGalW insertions were used (410.2, 410.17, 410.N13 and 410.N2b). In addition we also used the X chromosomal P element line 109C1 (obtained from Ed Giniger) for part of the screen. Two stocks that carry a stable genomic transposase source were used: P[ry+, Δ2-3] TM3 / CxD and H[w+,Hop2] CyO / Sp. The 403.9+14 stock carries *UAS-GFP* transgenes on the 3rd chromosome.

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