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# Systematic gain-of-function genetics in *Drosophila*

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## SUMMARY

**A modular misexpression system was used to carry out systematic gain-of-function genetic screens in *Drosophila*. The system is based on inducible expression of genes tagged by insertion of a P-element vector carrying a GAL4-regulated promoter oriented to transcribe flanking genomic sequences. To identify genes involved in eye and wing development, the 2300 independent lines were screened for dominant phenotypes. Among many novel genes, the screen identified known genes, including *hedgehog* and *decapentaplegic*, implicated in these processes. A genetic interaction screen for suppressors of a cell migration defect in a hypomorphic *slow border cells* mutant identified known genes with likely roles in tyrosine kinase signaling and control of actin cytoskeleton, among many novel genes. These studies demonstrate the ability of the modular misexpression system to identify developmentally important genes and suggest that it will be generally useful for genetic interaction screens.**

Key words: Disc development, Cell migration, Gal4, Misexpression, Suppression screen, *Drosophila*

## INTRODUCTION

The ability to screen or manipulate the genomes of model organisms provides a powerful means to analyze complex biological processes. A variety of genetic methods have been developed to identify genes and to link them to their biological functions. In model organisms like yeast or *Drosophila* and in plants these include large scale screening for mutations. The utility of this approach is best exemplified by screens for genes affecting cell cycle in yeast or embryonic development in flies and plants (Hartwell et al., 1974; Nüsslein-Volhard and Wieschaus 1980; Jurgens et al., 1994). The principal advantage of this approach is that it allows analysis of genes important for a particular process with no requirement for prior knowledge about their identity or molecular nature.

In conventional genetic screens, genomes are mutagenized using chemicals or ionizing radiation to create random mutations; transposable elements have come into use as mutagenic agents because they facilitate cloning of the affected gene (Garfinkel et al., 1988; Bancroft et al., 1992; Spradling et al., 1995). Each of these mutagens typically produces mutations that reduce or eliminate gene function. However, it has long been recognized that rare gain-of-function mutations can be extremely informative about gene function. Gain-of-function mutants have been isolated fortuitously as mutants that are recognized by dominant phenotypes (notable examples include homeotic mutations in *Drosophila* (Lewis 1978; Gehring 1985) or as rare induced mutations that alter or increase gene activity (e.g. the *sevenmaker* allele of *rolled*; Brunner et al., 1994).

Forced expression of genes is a useful means to screen for particular functions: well known examples include expression cloning of genes involved in embryonic patterning in *Xenopus* (Cho et al., 1991; Smith and Harland 1992; Lemaire et al., 1995; Bouwmeester et al., 1996) and conversion of fibroblasts to myoblasts by forced expression of MyoD (Davis et al., 1987). In addition, gene over-expression with multi-copy plasmid libraries has been successfully used as a method for carrying out genetic suppression screens in yeast (Bender and Pringle 1989; Ramer et al., 1989). Such screens are based on the observation that increased expression of one gene can suppress the phenotype of a mutation in another gene, suggesting that their products are involved in the same process.

It has been estimated that over 2/3 of all *Drosophila*, *C. elegans* and yeast genes show no obvious loss-of-function phenotypes when mutated (Sulston et al., 1992; Dujon et al., 1994; Miklos and Rubin, 1996), perhaps due to functional redundancy. Over- and misexpression screens may provide a means to identify roles for the products of such genes.

To allow systematic misexpression screens in *Drosophila*, Rørth (1996) developed a modular system combining P-element insertional mutagenesis with GAL4 regulated gene expression (Brand and Perrimon 1993). The system is designed to allow conditional expression of genes that are randomly tagged by insertion of a 'target' P-element (see Fig. 1). The target P-element carries GAL4 binding sites and a basal

promoter oriented to direct expression of genomic sequences adjacent to the P-element insertion site. When combined with a source of GAL4, the P-element will direct expression of any gene that happens to lie next to its insertion site.

Here we report the application of the modular misexpression system in two different types of genetic screen: a simple gain-of-function screen and a suppression screen. The gain-of-function screen was designed to identify genes involved in wing and eye development by GAL4 driven expression in otherwise wild-type genetic background. Along with a collection of previously unknown genes, the screen identified several known genes (including *hedgehog* and *decapentaplegic*) with important functions in wing and eye development. The suppression screen was designed to identify genes that, when over-expressed, suppress the cell migration defect of a hypomorphic mutation in the *slow border cells* (*slbo*) gene. Among the suppressors, genes encoding proteins with known roles in axonal outgrowth and actin-cytoskeleton remodeling were found, consistent with a role in cell migration. Together, these studies suggest that systematic over-expression of genes can be used to identify genes involved in a variety of biological processes.

## MATERIALS AND METHODS

### The EP collection

The EP element is described in Rørth (1996). To make the EP collection, an EP element was mobilized from CyO by crossing with a stable source of P transposase (Robertson et al., 1988). 2300 independent new inserts were mapped to chromosomes X, 2 or 3 and balanced for further analysis. Previous analyses had suggested that P-element insertional specificity was different in the male versus female germline (Zhang and Spradling, 1993). To maximize chances of recovering diverse insertion sites, mobilization was therefore carried out in the female (30% of new jumps) as well as the male (70% of new jumps) germline. Characterization of the entire collection of EP lines by the Berkeley Drosophila Genome Project will include cytological mapping and sequencing of flanking DNA. These data and the EP collection will be available through the genome project (http://fruitfly.berkeley.edu/).

### Molecular characterization of EP lines

To characterize the genes identified by gain-of-function phenotypes, flanking DNA was isolated by plasmid rescue and the genomic region immediately downstream of the EP element promoter (at the 3′ end of the P-element) was sequenced for 60/60 lines identified in the *slbo* suppressor screen and for 31/163 lines identified in the ombGAL4 screen. The 31 EP lines from the ombGAL4 screen were picked as interesting, based on phenotypic analysis including use of other GAL4 drivers. A minimum of 25 base pair DNA flanking the 5′ end of the P-element was sequenced by inverse PCR for 87/119 lines identified in the dppGAL4 and sevGAL4 screens. Because the inverse PCR sequences are on average short and are directed in the opposite orientation from the sequences transcribed by the EP element these sequences will identify known genes at a lower relative frequency than the sequences from plasmid rescued 3′ ends of the EP inserts.

## RESULTS

### Gain-of-function screen strategy

The target P-element vector is designed so that GAL4-dependent transcription begins within the P-element and extends out into the flanking genomic DNA (Fig. 1A). Target P-element insertion lines are designated ‘EP’ to denote that the P-element contains both the enhancer (GAL4 binding sites) and the promoter that are used to direct transcription of flanking sequences. The screening strategy takes advantage of the fact that P-elements insert preferentially into 5′ ends of genes (Spradling et al., 1995) allowing EP elements to direct expression of essentially full-length transcripts from adjacent genes (Rørth 1996). However, it is also possible to generate antisense transcripts if an element inserts into the transcribed sequences of a gene in antisense orientation (see below). Unless otherwise stated, the relevant EP element is positioned such that it can drive full-length or near full-length sense transcription of all genes identified and discussed in the following.

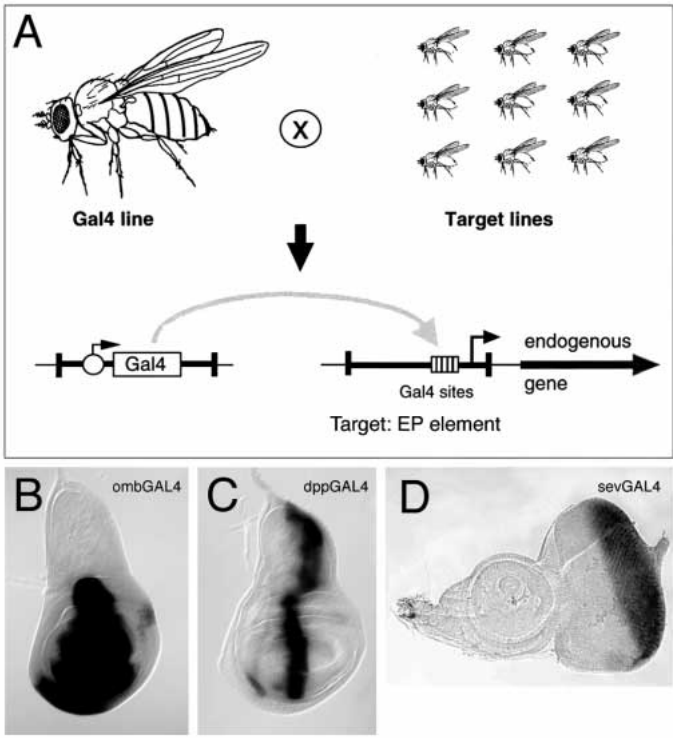


**Fig. 1.** Outline of the modular misexpression screen. (A) Target lines each contain a single EP target element at an unknown position in the genome. When mated with GAL4 flies, progeny will contain both elements as shown below. This allows GAL4 to bind its sites within the EP element and thereby induce the EP promoter to transcribe the gene immediately adjacent to the element. (B-D) Examples of expression patterns in GAL4 lines. Expression of (B) ombGAL4, (C) dppGAL4 and (D) sevGAL4 respectively in third instar wing (B,C) and eye (D) imaginal discs. The larvae also contained a UAS-lacZ transgene and β-gal expression was visualized by X-gal staining.

### Dominant patterning phenotypes

A collection of 2300 independent EP insertion strains was generated to allow systematic misexpression of adjacent genes by crossing them to different GAL4 drivers. To identify genes affecting wing development, ombGAL4 and dppGAL4 were used to drive EP expression in the wing imaginal disc. ombGAL4 is expressed in a broad region of the wing disc covering most of the presumptive wing blade (Fig. 1B; Lecuit

**Table 1. Summary of screen results**

| | ombGal4 | dppGal4 | sevGal4 | slboGal4 |
|---|---|---|---|---|
| Scored positive | 163 (7%) | 47 (2%) | 98 (4%) | 60 (3%) |
| Overlap (omb) | 163/163 | 28/163 | 48/163 | 2/163 |
| Overlap (dpp) | 28/47 | 47/47 | 28/47 | 0/47 |
| Overlap (sev) | 48/98 | 28/98 | 98/98 | 2/98 |
| Overlap (slbo) | 2/60 | 0/60 | 2/60 | 60/60 |
| Phenotypic classes (%) | Lethal 12% | Lethal 17% | Lethal 6% | Migration in slbo ovaries |
| | Vein defects 26% | Vein defects 25 % | Mild rough eye misalignment 37% | 3-4 fold ↑ 62% |
| | Scalloping 42% | Scalloping 6% | Glassy/fusion 47% | 4-8 fold ↑ 35% |
| | Growth 23% | Scutellum 53% | Reduced eye 11% | 13-17 fold ↑ 3% |
| | Other 17% | Other 21% | | |

"Scored positive" indicates number of EP lines producing any phenotype with the indicated GAL4 driver (% of total indicated in parenthesis). Overlap indicates EP lines scored positive in two screens. The denominator is the number of lines scored positive with the GAL4 driver indicated in parenthesis. The numerator is the number of lines which also scored positive with the GAL4 driver in the column. Phenotypic classes for dppGAL4, SevGAL4 and ombGAL4 are described in the main text. Classification may add up to more than 100% as some EP lines show multiple phenotypes. A subset of the EP lines producing scalloping phenotypes only do so with ombGAL4, but not with other drivers tested (dppGAL4, ptcGAL4, enGAL4). Since ombGAL is a hypomorphic mutant allele of *omb* (Lecuit et al., 1996), some of these may reflect specific genetic interaction with *omb.* Quantification of migration in *slbo* ovaries is described in Fig 4.

**Table 2. Genes identified**

| ombGal4 | dppGal4 | sevGal4 | slboGal4 | EP number(s) |
|---|---|---|---|---|
| escargot | escargot | escargot | - | 633; 684; 2009; 2159 |
| hedgehog | hedgehog | hedgehog | - | 3521 |
| dpp | - | dpp | - | 2232 |
| patched | - | - | - | 941 |
| yan | - | - | - | 598 |
| - | - | scalloped | - | 1435 |
| DSP1 | - | DSP1 | - | 355 |
| misshapen | - | - | - | 549 |
| - | - | - | hsp27 | 329 |
| - | - | - | Abelson TK | 3101 |
| - | - | - | α-Adaptin | 2519 |
| - | - | - | big brain | 2278 |

Most of the previously known genes identified in our screens are listed, according to the screen in which they were isolated. Note that the number of genes identified at present will be an underestimate because not all EP lines have been sequenced (see materials and methods for details). In addition, a significant number of EP lines directed expression of genes identified as either P-element insertion lethals or as expressed sequence tags (5′ ends of cDNAs) by the Berkeley Drosophila Genome Project. *misshapen* encodes a protein kinase with similarity to ste 20 kinase (Treisman et al., 1997). Lack of function mutations perturb photoreceptor cell shape and orientation. Big brain was isolated as a neurogenic gene, required for correct selection of cell fate. The remaining genes are discussed in the main text.

et al., 1996). dppGAL4 is expressed in a narrower stripe of cells in the center of the wing disc (Fig. 1C; Staehling-Hampton et al., 1994; Masucci et al., 1990). To analyze eye development, SevGAL4 was used to direct EP expression in cells posterior to the morphogenetic furrow of the eye disc (Fig. 1D; Basler et al., 1989; Bowtell et al., 1989).

Table 1 summarizes the results of the gain-of-function screens for phenotypes in wing and eye. 7% of all EP lines (163/2300) produced a detectable phenotypic abnormality when expressed under control of ombGAL4; as compared with 2% for dppGAL4 and 4% for sevGAL4. A number of EP lines caused lethality in combination with these GAL4 drivers, presumably reflecting the fact that they also drive expression in larvae outside the wing or eye disc and/or earlier in development. However, we mostly identified visible phenotypes. In the wing, ombGAL4 and dppGAL4 produced phenotypes including defects in the pattern or differentiation of veins (venation defects); nicking of the wing margin (scalloping) or other less easily defined defects (grouped as 'other'). In addition, ombGAL4 produced a class of growth defects in which wings exhibited either abnormal over-growth or undergrowth. This class includes alterations in growth that result from alterations in wing pattern as wells as well as alterations in size without obvious pattern abnormality. dppGAL4 produced a class of defects in the thoracic body wall, a region where ombGAL4 is not expressed. This class is scored as scutellum defects, indicating malformation or abnormal bristle pattern.

In general we observed more severe defects when EP lines were expressed under ombGAL4 control than when expressed under dppGAL4 control. This may be due to ombGAL4 driving higher levels of expression than dppGAL4 as well as its broader domain of expression (Fig. 1B,C). Differences in GAL4 expression levels may also explain why a larger number of EP lines give a dominant phenotype with ombGAL4 than with dppGAL4. Approximately half of the EP lines that scored positive with dppGAL4 did not score positive with ombGAL4, possibly because dppGAL4 is expressed in the thoracic body wall where ombGAL4 is not expressed.

Four percent of EP lines produced phenotypes under sevGAL4 control. The visible phenotypes varied in severity from mildly rough eyes with misaligned ommatidia to very rough or glassy eyes with ommatidial fusions. In a few cases, the eye was clearly reduced in size. Half of the lines scored positive with sevGAL4 were also positive with ombGAL4 and a third were also positive with dppGAL4. This relatively high degree of overlap between lines affecting eye and wing development was expected, as many well characterized genes are known to regulate development of both these structures.

## Known genes identified in the dominant phenotype screen

Localized expression of the secreted signaling molecules Hedgehog, Wingless and Dpp is critical for normal wing and eye development (Heberlein et al., 1993; Basler and Struhl 1994; Capdevila and Guerrero 1994; Diaz-Benjumea and Cohen 1995; Treisman and Rubin 1995), so it was anticipated that a screen based on misexpression of genes might identify these genes or components of their signal transduction pathways. Among the genes identified in screens using ombGAL4, dppGAL4 and sevGAL4 we found *hedgehog*, *patched* and *dpp* (Table 2; Fig. 2). Misexpression of *dpp* (EP2232) by ombGAL4 produced defects in wing growth and vein pattern (Fig. 2C), similar to those described previously when Dpp was broadly misexpressed (Capdevila and Guerrero 1994; Ingham and Fietz 1995). Misexpression of *hedgehog* was

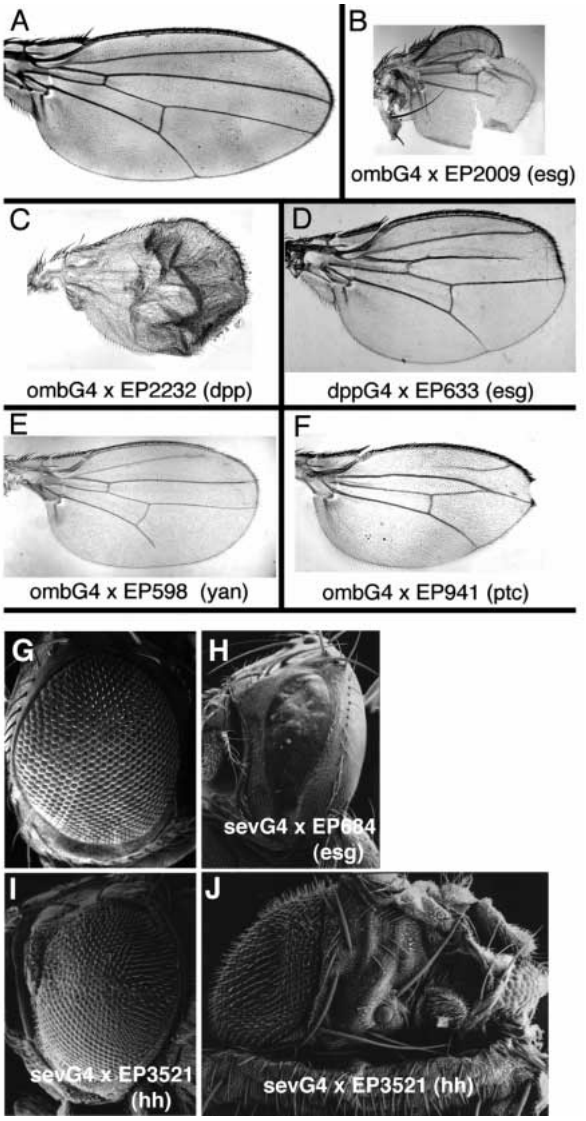


**Fig. 2.** Dominant phenotypes in wing and eye. (A) Wild-type wing. (B) ombGAL4 × EP2009 wing. EP2009 directs *escargot* (*esg*) expression. (C) ombGAL4 × EP2232 wing. EP2232 directs *dpp* expression. (D) dppGAL4 EP633 wing. EP933 directs *esg* expression. (E) ombGAL4 × EP598 wing. EP598 directs *yan* expression. (F) ombGAL4 × EP941 wing. EP941 directs *patched* (*ptc*) expression. All wings are shown at comparable magnification. Note that all wings from ombGAL4 are from female flies. ombGAL4 is a hypomorphic mutant allele of omb. ombGAL4 males show scalloping. (G) Scanning electron micrograph of wild-type eye. (H) SevGAL4 × EP684 eye, EP684 directs *escargot* (*esg*) expression. (I,J) SevGAL4 × EP3521 eye. EP3521 directs *hedgehog* (*hh*) expression. Escaper adults showed variable phenotypes from mildly rough eye (I) to reduced (J) or missing eye and head structures.

lethal with ombGAL4 and dppGAL4 and semilethal with sevGAL4, but produced occasional escapers with rough or reduced eyes (Fig. 2I,J). Misexpression of *patched* (EP941) by ombGAL4 produced wings with reduced size, particularly in the region between veins 3 and 4 veins (Fig. 2F). This resembles the phenotype previously described for over-expression of *patched* (Johnson et al., 1995). Patched functions to limit the level and range of Hh activity in the wing disc (Chen and Struhl 1996), so overexpression of Patched produces a phenotype resembling a reduction of Hedgehog activity.

All three gain-of-function screens identified the *escargot* gene. Overexpression of *escargot* by sevGAL4 produces small glassy eyes (Fig. 2H) and causes a reduction in the size of the wing with ombGAL4 or dppGAL4. Using dppGAL4, mild reduction is observed in the region between veins 3 and 4, where dpp is expressed; using ombGAL4 more extensive reduction was observed (Fig. 2B,D). Using other GAL4 drivers we noted that reduction of growth in the wing is restricted to the region in which *escargot* is overexpressed (not shown). Although *escargot* has been implicated as a negative regulator of endoreduplication in larval and imaginal cell cycles (Hayashi et al., 1993, Hayashi 1996) it is not entirely clear why its overexpression in diploid tissue should cause undergrowth.

The ombGAL4 screen identified *yan* on the basis of a reduced wing phenotype (Fig. 2E). The wing is small and shows mild vein pattern defects. *yan* encodes an ETS domain protein that is regulated by *Drosophila* MAP Kinase and implicated in cell fate determination (O'Neill et al., 1994; Rebay and Rubin, 1995). *yan* is required for photoreceptor development, but in agreement with previous results (Rebay and Rubin, 1995), overexpression of wild-type *yan* under control of the sevenless enhancer did not perturb eye development. Similarly, *scalloped* was identified only in the sevGAL4 screen. *scalloped* is required for wing development but is already expressed in a broad domain in the wing disc (Campbell et al., 1992).

Dorsal switch protein (DSP1), a protein which converts the activator Dorsal into a repressor at specific DNA sites (Lehming et al., 1994), produced phenotypes in both the wing and the eye. DSP1 interacts with proteins of the rel family, which in *Drosophila* have been implicated in immunity as well as dorsal/ventral patterning. Based on its broad activity in vitro (Kirov et al., 1996), it is possible that DSP1 act as a repressor of other transcription factors in eye and wing discs.

### Suppression of the migration defect in *slow border cells* mutants

During *Drosophila* oogenesis, 6-8 specialized somatic follicle cells, called border cells, delaminate from the follicular epithelial sheet and migrate to a specific place next to the oocyte (Fig. 3B). The *Drosophila* C/EBP transcription factor, encoded by the *slow border cells (slbo)* locus, is specifically expressed in border cells and is required for their migration (Montell et al., 1992). In *slbo* mutant ovaries, border cells migrate late or not at all depending on the strength of the allele. As a result, the *slbo* mutant females are sterile.

The *slbo* phenotype suggests that C/EBP regulates genes required for cell migration. To address this possibility we used the modular misexpression system to screen for second site genetic suppressors that restore fertility in *slbo* mutant females when expressed in the border cells. Suppressors could be genes normally activated by C/EBP in border cells or other genes which (in this situation) are rate-limiting for cell migration. Note that because the *slbo* mutant used is a hypomorph which retains some C/EBP activity, it is only necessary to increase border cell migration to an extent that restores detectable fertility.

In order to drive EP expression in border cells, we first

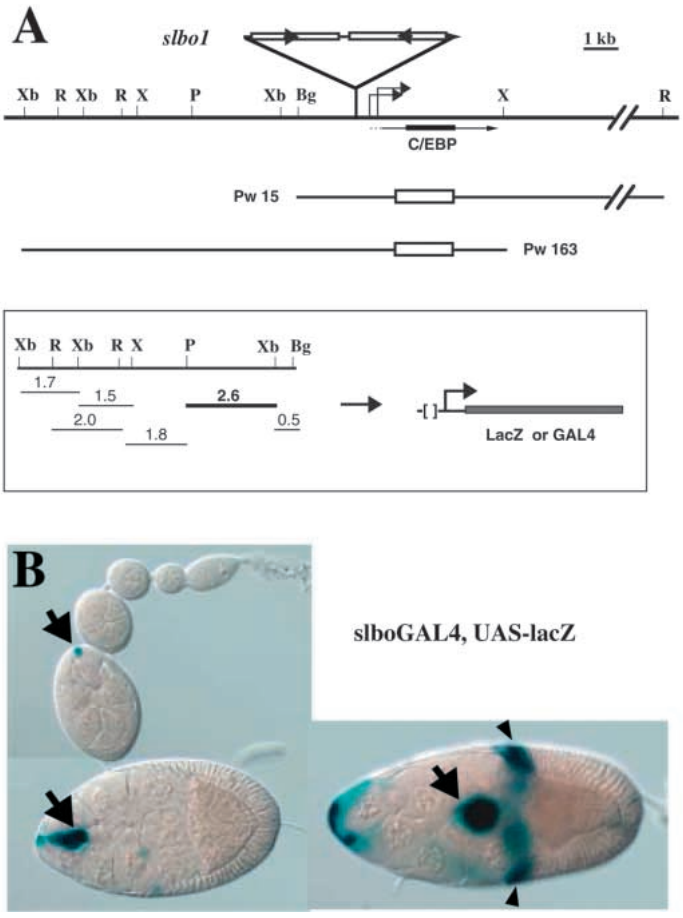


**Fig. 3.** Contructing and testing slboGAL4. (A) A genomic map of the *slbo* locus, including the position of the *slbo*$^{1}$ enhancer trap insertion (Montell et al., 1992), is shown at the top. The Pw15 transgene depicted below rescues the *slbo* (C/EBP) null lethal phenotype and drives expression in embryos (Rørth, 1994), but not in the ovary. The Pw163 transgene rescues the *slbo* female sterile phenotype and drives expression indistingishable from that of C/EBP (*slbo*) in ovaries (P. R. unpublished). Strategy for identifying the *slbo* enhancer is shown in the box. The 2.6 kb fragment had border cell enhancer activity and was cloned upstream of the hsp70 minimal promoter, GAL4 coding region and hsp70 3′ end in pCasper to make slboGAL4. (B) Expression driven by slboGAL4 in the ovary. Expression is visualized by a UAS-lacZ construct carrying an enhancer/promoter used in the EP element (Rørth, 1996). Arrows point to border cells before and after migration. Small arrowheads point to centripetal follicle cells in a stage 10 egg chamber (these cells also express C/EBP). Transgenic flies were obtained by P-element mediated transformation and ovaries were stained with X-gal to reveal the expression pattern of β-gal.

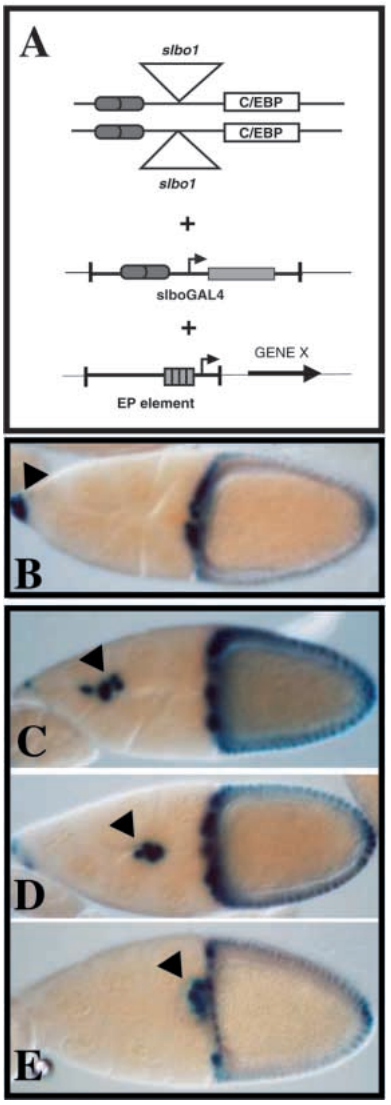


**Fig. 4.** Quantification of migration in *slbo* ovaries. The genotype of females tested in the *slbo* suppression screen is shown schematically in A. The *slbo*$^{1}$ allele is an enhancer trap insertion. This allows visualization of the border cells by X-gal staining as shown in B-E (arrowheads indicate border cells). B-E show stage 10 egg chambers with no, $<\frac{1}{2}$, $>\frac{1}{2}$ and complete migration, respectively. Control females (no EP insert) showed 3.4% chambers of the C and D type but no completed migration and were sterile. EP suppressors of the sterile phenotype showed between 9 and 58% chambers of the C, D and E type (see Table 1).

constructed the slboGAL4 vector. Genomic DNA fragments from the region 5′ to the C/EBP transcription unit were cloned upstream of *lacZ* (Fig. 3A). One fragment (slbo2.6) resulted in expression in a pattern very similar to endogenous C/EBP in the ovary: in border cells and centripetal follicle cells. The slbo2.6 fragment was then placed upstream of GAL4 to make slbo-GAL4. *lacZ* expression in ovaries of females carrying slbo-GAL4 and a UAS-lacZ was similar to that of slbo2.6-lacZ, but with relatively higher expression at late stages of oogenesis (Fig. 3B). As a positive control for the suppression screen, we made flies harboring a UAS-C/EBP transgene and combined this with slbo-GAL4 in a homozygous *slbo*$^{1}$ mutant background (Montell et al., 1992). As expected, the *slbo* border cell migration defect was rescued (data not shown).

To identify *slbo* suppressors, 2082 EP lines were crossed into the *slbo*$^{1}$ mutant background in the presence of slbo-GAL4 and females containing both elements were tested for fertility (Fig. 4A). At 25°C, the *slbo*$^{1}$ mutant females with slbo-GAL4 but no EP insert were sterile. 4% of EP inserts restored detectable fertility in the initial screen. These lines were retested and migration was quantified as a percentage of stage 10 egg chambers showing detectable border cell migration (at least 100 egg chambers were scored; Fig. 4B). *slbo* mutant females with no EP insert show 3.4% migration, a level not sufficient for fertility. Of the initial 88 putative suppressors, 60 resulted in migration in at least 9% of egg chambers (an arbitrary cut off). The majority of these showed a moderate (but reproducible) 3- to 5-fold increase relative to control; a few had much more robust effects (Table 1). Most (56) EP inserts did not increase cell migration in the absence of slbo-GAL4, indicating that suppression of the *slbo* phenotype was indeed due to GAL4 driven overexpression of an endogenous gene.

EP inserts that suppress the *slbo* phenotype showed almost no overlap with those identified in the gain-of-function wing and eye screens (Table 1).

### Known genes identified in the *slbo* suppressor screen

Six of the EP suppressors were inserted such that they would direct essentially full-length transcripts of previously identified genes (Table 2). For each of these genes, a transgene with the corresponding cDNA cloned into the pUAST vector (Brand and Perrimon 1993) was able to reproduce the *slbo* suppressor effect in the presence of slbo-GAL4. This confirms that we have identified genes which suppress the migration defect in *slbo* mutant border cells when overexpressed.

Actin cytoskeleton remodeling is a key process in cell migration (Mitchison and Cramer, 1996). Two of the identified *slbo* suppressors (Table 2), Abl and hsp27, encode proteins which may control this process. Identification of Abelson non-receptor tyrosine kinase (*abl*) as a *slbo* suppressor was intriguing for several reasons. Loss-of-function mutations in *Drosophila abl* have been shown to decrease axonal outgrowth when activity of the *disabled* gene is also reduced (Gertler et al., 1989). A conserved role of these proteins in cell migration and pathfinding is suggested by recent reports that mouse *disabled* mutants exhibit defects in neuronal migration (Sheldon et al., 1997; Howell et al., 1997). In addition, Abelson can phosphorylate, and interacts genetically with, a protein of the VASP family called Enabled (Gertler et al., 1995). Mena, the mouse homolog of Enabled, has been directly implicated in actin polymerization associated with actin-driven movement of Listeria in cells (Gertler et al., 1996).

Receptor tyrosine kinases (RTKs) appear to regulate many developmentally controlled cell migrations, for example migration of neural crest cells (Wehrle-Haller and Weston, 1997). In *Drosophila*, the breathless RTK is required for tracheal cell migration (Klambt et al., 1992) and increased levels of the protein can suppress the *slbo* phenotype (Murphy et al., 1995). To test whether the ability of Abelson to increase border cell migration was a non-specific effect of increased tyrosine kinase activity, we expressed *Drosophila* Src64B (Kussick et al., 1993), another non-receptor tyrosine kinase in border cells using slbo-GAL4. This did not suppress the *slbo* phenotype (data not shown), arguing that the suppressor activity of Abelson was specific.

Alpha-adaptin was also identified as a *slbo* suppressor. Alpha-adaptin is a component of the AP2 adaptor complex of clathrin-coated pits at the plasma membrane; and mutations in *Drosophila* α-adaptin inhibit endocytosis (Gonzalez-Gaitan and Jackle 1997). Increased levels of α-adaptin protein may modulate receptor mediated endocytosis and thereby RTK signaling.

The functions of small heat shock proteins such as HSP27 are not well understood. They are likely to protect cells from the effects of heat and other stress, but also appear to function during normal development (reviewed by Arrigo and Landry, 1994). Traditional loss-of-function genetic analysis of small HSP function, for example in *Drosophila*, is hampered by there being several closely related small HSPs. These proteins are likely to overlap in function and thus show genetic redundancy. Tissue culture experiments indicate that small HSPs can directly affect the actin cytoskeleton (Lavoie et al., 1993). HSPs are phosphorylated in response to mitogen stimulation and RTK signaling (Zhou et al., 1993); the phosphorylation sites are important for the effects on the actin cytoskeleton (Lavoie et al., 1995). It is therefore tempting to speculate that small HSPs serve as a link between RTK signaling and the cytoskeletal remodeling required for cell mobility. The ability of hsp27 overexpression to suppress the *slbo* migration phenotype is consistent with such a role and we plan to investigate this in more detail.

### Induced loss-of-function mutations

In the EP element, GAL4-induced transcription is initiated within the P-element (Rørth, 1996). Thus, depending on the insertion site and orientation of the EP element relative to the transcription start site of the target gene, the EP element may direct either sense or antisense transcripts. Antisense transcripts could produce GAL4-dependent loss-of-function phenotypes. In a pilot screen, most inserts found near cloned genes or cDNAs were positioned and oriented to produce essentially full-length sense transcripts (Rørth, 1996). This was also true for all genes listed in Table 2 as well as a number of other known genes and ESTs. In a few lines, however, the EP element had inserted downstream of the transcription start site and in an orientation such that GAL4 induction would produce an antisense transcript.

One putative ‘antisense’ line, EP 2585, was isolated in the *slbo* suppression screen. This was one of the few suppressors with a positive effect on migration even in the absence of GAL4. Thus the EP insertion may cause a loss-of-function mutation, which (in a heterozygote) weakly suppresses the *slbo* phenotype. The suppression was greater in the presence of GAL4, which further suggested that an antisense transcript was induced and caused a more severe loss-of-function mutation. In the eye and wing gain-of-function screens, we identified one antisense insert in the *escargot* gene. The single antisense insert (EP683) produced a different mutant phenotype than the four independent sense inserts at the same gene. Taken together, these observations suggest that antisense loss-of-function phenotypes can be produced with this method, although they appear relatively infrequently.

## DISCUSSION

### Gain-of-function genetics

The results reported here demonstrate the utility of gene overexpression as a method for systematic genetic screening in *Drosophila*. The simple dominant gain-of-function screens identified a number of known genes implicated in pattern formation as well as a larger number of unknown genes. The gain-of-function phenotypes could either result from expression of a gene in the wrong place or in the wrong amount. *hedgehog* and *dpp* were identified because of spatial misexpression. Localized expression is important for their normal function. *patched* was identified because overexpression reduces Hedgehog signaling. Several of the novel genes do not show localized endogenous expression, but nonetheless alter growth and patterning when expressed under GAL4 control (data not shown). In these cases the gain-of-function effects are likely to be due to overexpression. Other

examples have been noted previously for components of signal transduction pathways, including activation of RTKs by overexpression (Di-Fiore et al., 1987) and activation of the wingless signal transduction pathway by overexpression of *dishevelled* (Axelrod et al., 1996; Neumann and Cohen 1996).

The identification of genes with known roles in patterning and growth control shows that the screen is capable of selecting the desired type of gene. However, it is important to consider that inappropriate expression of random proteins can perturb development for a variety of reasons. Abnormalities could result from entirely non-specific effects, for example reduced cell viability or failure of cells to differentiate appropriately. A potentially more informative class of defects are those that could result from overexpression of a protein which fortuitously interacts with an endogenous protein in a way that reduces its activity (e.g. titration by binding). Although the ectopic protein may not have a normal function in the tissue or process under study, it may nevertheless exert a specific dominant negative effect by compromising the function of an endogenous component. This scenario illustrates one way in which a gain-of-function genetic approach could provide unique molecular access to a regulatory pathway.

Gain-of-function genetics can also uncover functional information about genes which are not easily accessible through conventional lack-of-function approaches. Many genes are known not to have lack-of-function phenotypes under normal conditions. In some cases this may be due to functional redundancy through the presence of multiple copies of closely related sequences. As illustrated by the identification of HSP27, such genes can be identified in our screens. Another of the identified genes, Abelson tyrosine kinase, shows partial functional redundancy with several structurally dissimilar genes during embryogenesis (Gertler et al., 1989).

### Comparison of suppressor and dominant phenotype screens

Artifactual phenotypes should be less of a concern in suppression screens than in simple overexpression screens. Suppressor screens are expected to select for genes or specific gene functions that are normally important for the process under study because they seek to restore wild-type cellular or developmental functions. This seems particularly likely in screens such as the one performed here for improved cell migration in a *slbo* hypomorphic mutations. In this case the phenotype is caused by reduced activity of a transcription factor, so the defect probably reflects underexpression of particular target genes. Such target genes are good candidates to be among the suppressors identified by EP driven overexpression. We would expect these genes to be required for border cell migration, and consequently that lack-of-function mutations would reduce cell migration. Preliminary results indicate that several potent gain-of-function suppressors do in fact show decreased border cell migration as a lack-of-function phenotype.

Although it is difficult to predict a priori how much overlap there might be between gene products whose level of expression affects cell migration and those whose levels affect aspects of cell interaction involved in growth control and patterning, we find it encouraging that there is relatively little overlap between the very different sorts of screens. The screens do appear to identify different sets of genes.

### Genetic interaction screens

Suppressor screens of the sort described here are only one type of genetic interaction screen to which the misexpression system can be applied. Sensitized suppressor or enhancer screens performed by conventional EMS mutagenesis have proved to be a powerful tool for identifying new components of signal transduction pathways; for example the RAS pathway (Simon et al 1991; Rogge et al., 1991). In some cases such interaction screens have identified rare gain-of-function alleles (e.g. Brunner et al., 1994; Fortini and Artavanis-Tsakonas, 1994), suggesting that a systematic approach to gene over-expression may be useful.

Although the P-element based system described here was designed for use in *Drosophila*, our results suggest that application of comparable strategies might be of interest in other experimental systems. Retrotransposon-based vectors are currently used to generate insertional mutations in mice and zebrafish (Friedrich and Soriano, 1993; Gaiano et al., 1996), and could be modified to allow conditional misexpression of flanking genes. This would provide an important new tool for analysis of gene function in vertebrates.

### Genomic analysis

The available data on P-element insertion sites is primarily based on P-induced recessive lethal mutations. Most of these are caused by inserts in 5′ untranslated leader sequences of the mutated genes (Spradling et al., 1995). The EP collection represents an unselected pool of P-element insertions from which we have identified P-elements capable of producing phenotypes when expression of nearby genomic sequences is induced. Sequence analysis of the EP lines that express previously cloned genes or ESTs shows that 80% of these inserts lie upstream of the transcription start site, while only 20% have inserted within the 5′ UTR. This location is consistent with the requirement that the EP element directs near-full-length expression of the gene in which it has inserted, but no requirement for disrupting the gene. Further analysis of the EP collection by the Berkeley Drosophila Genome Project will help determine the true (unselected) P-element insertion site preference.

The screen succeeded in identifying a number of known players in the processes under study, but many more equally important known genes were not hit. This was expected as the screen was far from saturating. The *Drosophila* genome is estimated to contain 12000 genes (Miklos and Rubin, 1996). Since the EP element is directional, only 50% of insertions will be oriented so as to direct sense strand expression of the adjacent DNA. Combined with the observation that P-elements do not insert completely randomly in the genome (Spradling et al., 1995), this means that less than 10% of all genes are productively targeted in the 2300 EP lines screened. However, each of the screens identified a number of apparently very intriguing novel genes which are now under active investigation. Thus a more complete coverage of the genome by EP elements should allow an even larger number of interesting genes to be identified by this approach.

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