

# Targeted gene expression as a means of altering cell fates and generating dominant phenotypes

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## SUMMARY

We have designed a system for targeted gene expression that allows the selective activation of any cloned gene in a wide variety of tissue- and cell-specific patterns. The gene encoding the yeast transcriptional activator GAL4 is inserted randomly into the *Drosophila* genome to drive GAL4 expression from one of a diverse array of genomic enhancers. It is then possible to introduce a gene containing GAL4 binding sites within its promoter, to activate it in those cells where GAL4 is expressed, and to observe the effect of this directed misexpression on development. We have used GAL4-directed transcription to expand the domain of embryonic expression of the homeobox protein *even-skipped*. We show that

*even-skipped* represses *wingless* and transforms cells that would normally secrete naked cuticle into denticle secreting cells. The GAL4 system can thus be used to study regulatory interactions during embryonic development. In adults, targeted expression can be used to generate dominant phenotypes for use in genetic screens. We have directed expression of an activated form of the *Dras2* protein, resulting in dominant eye and wing defects that can be used in screens to identify other members of the *Dras2* signal transduction pathway.

Key words: targeted expression, enhancer trap, GAL4, cell fate

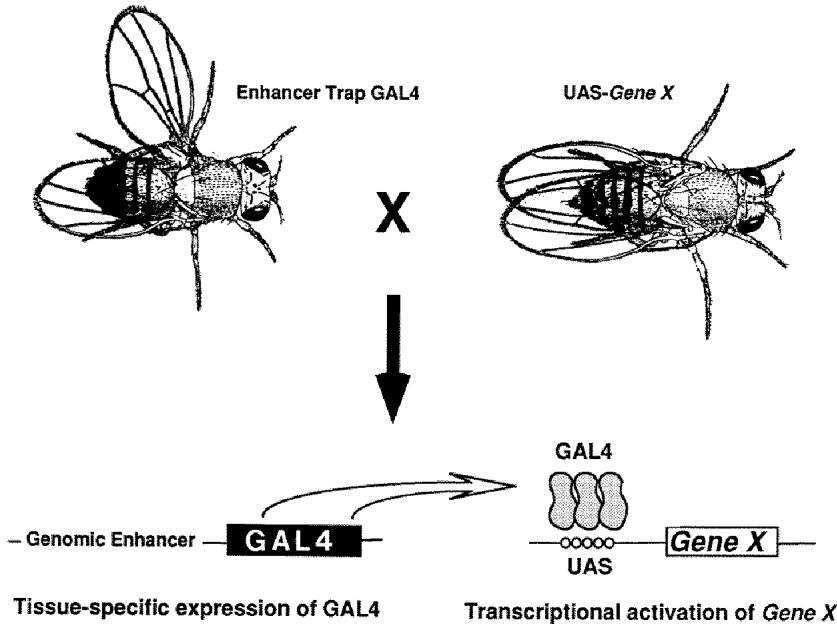
## INTRODUCTION

The ability to express a gene in a directed fashion is a useful means of analyzing its role in development. A switch in cell fate can often be induced by ectopic expression of a gene. The ability to alter transcription patterns at will would permit direct testing of models for cell fate determination. For example, misexpression of a ligand can be used to assay whether a receptor is restricted to specific cells or is present, and competent to respond to ligand-induced activation, in every cell of a particular tissue (such as the ability of cells expressing the *sevenless* receptor tyrosine kinase to respond to the presence of the ligand, *boss*; Van Vactor et al., 1991). A gene encoding a putative transcriptional activator (or repressor) can be expressed in a different cell, or at a different time of development, and its effect on the subsequent expression of a target gene assayed. In this way it is possible to determine if the activator is both necessary and sufficient for transcription of the target. Such an analysis is particularly useful where a protein acts as an activator in one context and as a repressor in another (as may be the case for the zinc-finger protein *Kruppel*; Sauer and Jackle, 1991).

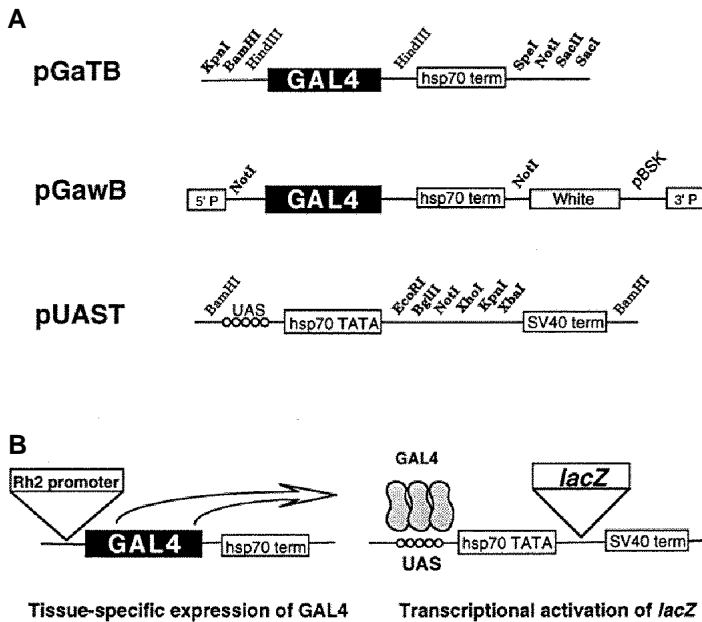
To date, two methods have been employed most widely to manipulate gene expression. The first is to drive expression of a gene from a heat shock promoter. The gene can then be turned on at a specific point in development by

heat shocking the transgenic animal (Struhl, 1985; Schneuwly et al., 1987; Ish-Horowitz and Pinchin, 1987; Ish-Horowitz et al., 1989; Gonzales-Reyes and Morata, 1990; Blochlinger et al., 1991; Steingrimsson et al., 1991). An advantage of the heat shock method is that it permits inducible expression; several disadvantages are that ectopic expression is ubiquitous, that basal levels of expression are observed from heat shock promoters, and that heat shock itself can induce phenocopies (Petersen and Mitchell, 1987; Petersen, 1990; Yost et al., 1990). The second technique is to drive expression of a gene using the transcriptional regulatory sequences from a defined promoter (Zuker et al., 1988; Parkhurst et al., 1990; Parkhurst and Ish-Horowitz, 1991). The use of tissue-specific promoters allows transcription to be restricted to a defined subset of cells, but is limited by the availability of cloned and characterized promoters that can direct expression in the desired pattern. Furthermore, if the gene product to be expressed is toxic to the organism, it is impossible to establish stable transgenic lines carrying the chimeric gene.

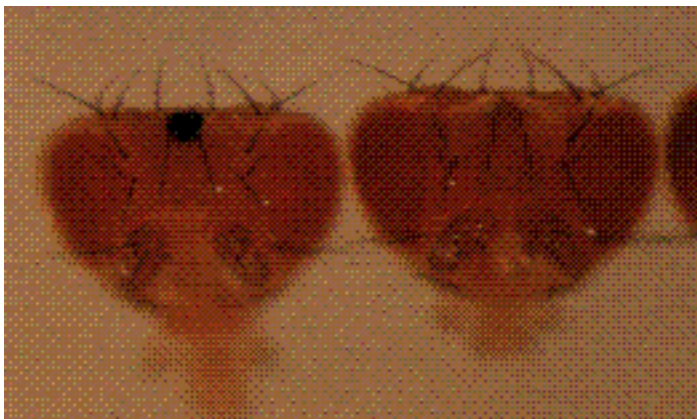
Here we describe a method for directing gene expression in *Drosophila* that overcomes these difficulties (Fig. 1). First, the system allows the rapid generation of individual strains in which ectopic expression of the gene of interest (the target gene) can be directed to different tissues or cell types. Second, the method separates the target gene from its transcriptional activator in two distinct transgenic lines.

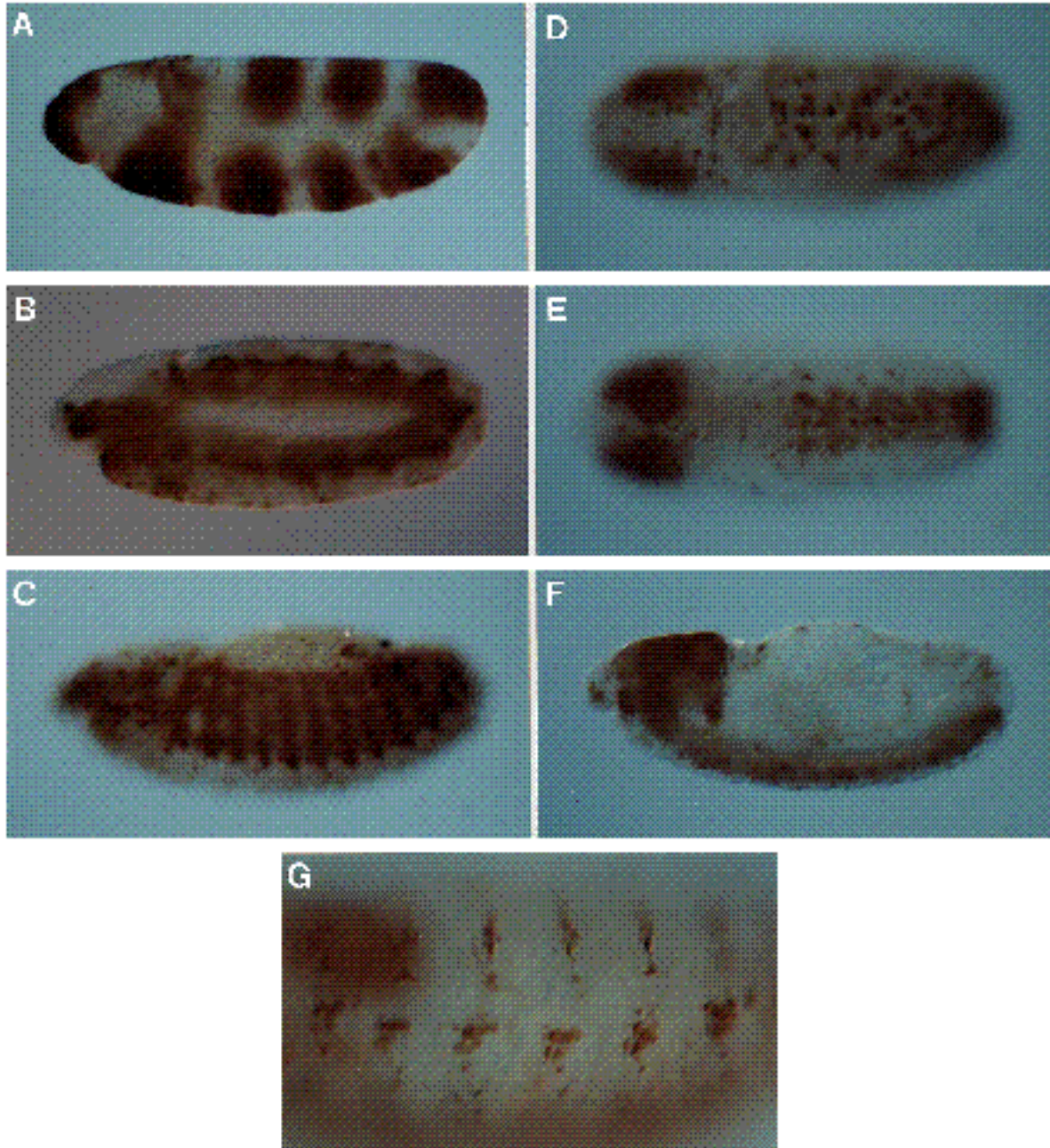


**Fig. 1.** Directed gene expression in *Drosophila*. To generate transgenic lines expressing GAL4 in numerous cell- and tissue-specific patterns, the GAL4 gene is inserted randomly into the genome, driving GAL4 expression from numerous different genomic enhancers. A GAL4-dependent target gene can then be constructed by subcloning any sequence behind GAL4 binding sites. The target gene is silent in the absence of GAL4. To activate the target gene in a cell- or tissue-specific pattern, flies carrying the target (*UAS-Gene X*) are crossed to flies expressing GAL4 (Enhancer Trap GAL4). In the progeny of this cross, it is possible to activate *UAS-Gene X* in cells where GAL4 is expressed and to observe the effect of this directed misexpression on development.



**Fig. 2.** (A) Vectors for directed gene expression. The vectors pGaTB, pGawB and pUAST are illustrated. To target GAL4 expression to specific cells, promoters can be subcloned upstream of GAL4 at the unique *Bam*HI site of pGaTB. pGawB is an enhancer detection vector that directs expression of GAL4 in a genomic integration site-dependent fashion. pUAST is designed to direct GAL4-dependent transcription of a gene of choice. The sequence is subcloned into a polylinker situated downstream of five tandemly arrayed, optimized GAL4 binding sites, and upstream of the SV40 small t intron and polyadenylation site. Unique restriction sites are indicated in bold letters. (B) GAL4-dependent expression of  $\beta$ -galactosidase in the ocelli. To direct expression of GAL4 to the photoreceptor cells of the ocelli, the promoter of the *Rh2* gene was subcloned upstream of GAL4 in pGaTB. Flies heterozygous for this gene construct (*Rh2-7-2*) were crossed to a line homozygous for the *UAS-lacZ* gene. In the progeny of this cross approx. 50% of the flies express  $\beta$ -galactosidase in the ocelli, as assayed by staining with X-Gal. On the left is the head of a fly that carries both the *Rh2-GAL4* fusion gene and the *UAS-lacZ* reporter construct, on the right is the head of a fly that carries only the *UAS-lacZ* construct.





**Fig. 3.** GAL4 expression patterns generated by enhancer detection. Three enhancer detection/GAL4 lines were crossed to a *UAS-lacZ* line (g4-1-2) and their progeny were assayed for expression of  $\beta$ -galactosidase by staining with anti- $\beta$ -galactosidase antibodies. (A) Line 1J3: pGawB has inserted at the *hairy* locus and activates expression of  $\beta$ -galactosidase in a pair-rule pattern of seven stripes, and in the head of a stage 11 embryo. (B) Line 24B: this GAL4 insertion directs expression of  $\beta$ -galactosidase first in the presumptive mesoderm, as shown in a stage 11 embryo, and then (C) in the muscle cells of a stage 13 embryo. (D) Line 31-1: this pGawB insertion directs expression of  $\beta$ -galactosidase in neuroblasts at stage 11 (D; dorsal view) and stage 12 (E; dorsal view), and then in neurons in both (F) the central and (G) the peripheral nervous system at stage 13. In all photographs anterior is to the left.

In one line the target gene remains silent in the absence of its activator, in the second line the activator protein is present but has no target gene to activate. This ensures that the parental lines are viable. Only when the two lines are crossed is the target gene turned on in the progeny, and the phenotypic consequences of misexpression (including

lethality) can be conveniently studied. Finally, the method is designed to generate lines that express a transcriptional activator, rather than an individual target gene, in numerous patterns. The target can then be activated in different cell- and tissue-types merely by crossing a single line carrying the desired target to a library of activator-expressing

lines. Thus, a library of different activator-expressing lines can direct each new target gene to be expressed in numerous distinct patterns.

A first step is to generate lines expressing a transcriptional activator in a variety of patterns. It is necessary to use a transcriptional activator that has no endogenous targets in *Drosophila*, so as to express ectopically only the gene of interest. An activator from yeast, GAL4, can activate transcription in flies but only from promoters that bear GAL4 binding sites (Fischer et al., 1988). The GAL4 protein is a potent transcriptional activator in yeast, and the protein has been extensively characterized with respect to both DNA binding and transcriptional activation (reviewed by Ptashne, 1988). The GAL4 binding site has been mutagenized, generating an optimized site to which GAL4 binds with high affinity (Webster et al., 1988). Numerous mutations have been made in the GAL4 transcriptional activation domain, producing activators of varying strengths (Ma and Ptashne, 1987a,b; Gill and Ptashne, 1987; Johnston and Dover, 1988). GAL4 has been shown to activate transcription, not only in *Drosophila*, but also in plants and in mammalian cells, again only from promoters that bear GAL4 binding sites (Fischer et al., 1988; Ma et al., 1988; Kaki-dani et al., 1988; Webster et al., 1988; Ornitz et al., 1991).

We have taken two approaches to generate different patterns of GAL4 expression. First, *GAL4* transcription can be driven by characterized *Drosophila* promoters. The second approach is based on the 'enhancer detection' technique, which was developed as a means of identifying transcriptional regulatory elements *in situ* in the *Drosophila* genome (O'Kane and Gehring, 1987; Bier et al., 1989; Bellen et al., 1989; Wilson et al., 1989). O'Kane and Gehring (1987) fused the *E. coli lacZ* gene to the weak P-transposase promoter, and showed that this reporter gene could respond to neighboring transcriptional regulatory elements. By fusing the GAL4 coding sequence to the P-transposase promoter, we constructed a vector that, depending upon its genomic site of integration, can direct expression of GAL4 in a wide range of patterns in embryos, larvae and adults. This eliminates the need to link numerous different promoters to the *GAL4* gene, and allows expression in novel patterns from enhancers that have not yet been described. In addition, the enhancer detection/GAL4 vector can be mobilized to new genomic sites simply by P-transposition (Cooley et al., 1988; Robertson et al., 1988). In this way, a single trans-formant can be used to generate a large number of trans-genics, each exhibiting a different GAL4 expression pattern.

To create GAL4-responsive target genes, we designed a vector into which genes can be subcloned behind a tandem array of five optimized GAL4 binding sites (hereafter referred to as the UAS, for Upstream Activation Sequences), and upstream of the SV40 transcriptional terminator. It is possible, then: (1) to subclone any sequence behind GAL4 binding sites; (2) to activate that target gene only within cells where GAL4 is expressed and (3) to observe the effect of this aberrant expression on development.

Using the GAL4 system we have been able to generate specific embryonic phenotypes by misexpression of the homeobox-containing transcription factor *even-skipped*. *even-skipped* is involved in determining cell fate, first

during embryonic segmentation, and later during the development of the central nervous system (CNS; Frasch et al., 1987, 1988; Doe et al., 1988). By restricting misexpression within the ectoderm, we have been able to alter epidermal cell fates specifically without affecting central nervous system development. This now permits the separate functions of *even-skipped* in different tissues to be studied independently of one another.

Dominant phenotypes can be generated in adults using the GAL4 system to restrict the expression of potentially lethal products, such as constitutively active proteins. Suppression or enhancement of these phenotypes can then be used to identify interacting gene products, or to establish epistatic relationships. Dominant mutations recovered by classical genetic techniques have proved invaluable in identifying and ordering the components of several signal transduction pathways in *C. elegans* and *Drosophila*. For example, the Ras proteins have been shown to act in determining cell fate in the nematode vulva and in the *Drosophila* compound eye (Han and Sternberg, 1990; Simon et al., 1991). Of the three Ras homologues isolated from flies (Neuman-Silberberg et al., 1984; Brock, 1987), *Dras1*, which is most similar to Ha-*ras*, appears to act downstream of the receptor tyrosine kinases *sevenless* and *DER* (the *Drosophila* EGF receptor) to determine cell fate during eye development (Simon et al., 1991). *Dras3* is also required in the eye and encodes a homologue of the human *rap1* gene, a possible Ras antagonist (Hariharan et al., 1991). *Dras2* is most similar to R-*ras* (Lowe et al., 1987), but its role in development has not yet been determined. We have generated dominant, visible phenotypes in adults by targeted expression of constitutively active *Dras2* (Bishop and Corces, 1988). Genetic suppression of these phenotypes can now be used to identify mutations in interacting gene products, as a means to elucidate the *Dras2* signal transduction pathway.

## MATERIALS AND METHODS

### GAL4 expression vectors

1. *pGATB* and *pGATN*: we have constructed vectors in which either a unique *NotI* or *BamHI* site is inserted upstream of the GAL4 coding region. The GAL4 coding sequence was excised from vector pLKC15 (a gift from L. Keegan) as a *HindIII* fragment. This fragment extends from a synthetic *HindIII* site inserted approximately 15 nucleotides upstream of the initiator methionine, and includes the complete GAL4 coding sequence and its transcriptional terminator (L. Keegan, personal communication). The *HindIII* fragment was subcloned in the vector pHSREM (Knipple and Marsella-Herrick, 1988) to give plasmid pF18-13, and the heat shock consensus sequences were removed from the promoter by digestion with *BglII* and *EspI*. A unique *BamHI* site was inserted in place of the heat shock boxes to create pGATB. To construct pGATN, a *NotI* site was inserted in place of the heat shock elements. Promoters can now be subcloned upstream of GAL4, and the fusion gene moved from the pHSREM vector backbone into a P-element vector.

2. *Rh2-GAL4*: the *Rh2* promoter (Mismer et al., 1988) was excised from vector pHS7 as a *BamHI* fragment and subcloned into the *BamHI* site of vector pGATB, creating p25/18XI. The *Rh2-GAL4* fusion gene was then subcloned, as a *KpnI-NotI* fragment, into pCaSpeR4 (a gift from C. Thummel and V. Pirrotta) to make pF72.

3. pGawB: as a first step in creating an enhancer detection GAL4 vector, we modified the enhancer detection *lacZ* vector, plwB (Wilson et al., 1989). To remove the *NotI* site in the vector, plwB was digested with *NotI* and the 5' overhanging ends were filled using T4 polymerase. The resultant blunt ends were then ligated to make plasmid p41-4. To remove the P-transposase-*lacZ* fusion gene, p41-4 was digested with *HindIII*, the *HindIII* fragment was removed and the plasmid was religated, forming p41-4-H3-1. A short linker oligonucleotide, formed by annealing the sequences: 5'-AGCTTGGTTAACGCGGCCGC-3' and 3'-ACCAATTGCGCCGCGTCGA-5', was then subcloned into the *HindIII* site of p41-4-H3-1. In the resultant plasmid, p41-4Hpa, the *HindIII* site is maintained and a unique *HpaI* site is introduced.

To reconstitute the 5' end of the P-element and the P-transposase promoter, we synthesized an oligonucleotide that extends from the *HindIII* site in the 5' end of the P-element to nucleotide 140, followed by the sequence CGGCCGC, to create a *NotI* site. The oligonucleotide was subcloned as a *HindIII*-blunt ended fragment into p41-4Hpa cut with *HindIII* and *HpaI*, to create p41-4Hpa14.

As a final step, the GAL4 coding sequence followed by the *hsp70* terminator was isolated from pGaTN by digestion with *NotI*. The *NotI* fragment was subcloned into the unique *NotI* site of p41-4Hpa14 to create pGawB.

### GAL UAS fusion genes

1. pUAST: we constructed a vector into which genes can be subcloned behind the GAL UAS. A fragment containing five optimized GAL4 binding sites (the 'ScaI site' 17-mer; Webster et al., 1988) and a synthetic TATA box (Lillie and Green, 1989) separated by a unique *NotI* site from the SV40 terminator was excised from pF40X2-1 by digestion with *NsiI* and *SpeI*. The fragment was subcloned into the P-element vector pCaSpeR3 (a gift from C. Thummel and V. Pirrotta), cut with *PstI* and *XbaI*, to give plasmid pF91. This vector was used to drive expression of *Dras2<sup>Val14</sup>*, but appeared to be inefficient in promoting transcription, and so was modified as follows.

pF91 was digested with *XbaI*, and a fragment spanning the TATA box was removed. This was replaced by a fragment containing the *hsp70* TATA box, generated as a PCR product using pCaSpeR-hs (a gift from C. Thummel and V. Pirrotta) as a template. The PCR product begins with an *NheI* restriction site, extends from eleven nucleotides upstream of the *hsp70* TATA box to the *SacII* site in pCaSpeR3, and is followed by restriction sites for *XhoI*, *KpnI* and *XbaI*. The resulting vector, named pUAST, consists of five tandemly arrayed, optimized GAL4 binding sites, followed by the *hsp70* TATA box and transcriptional start, a polylinker with unique restriction sites for *EcoRI*, *BglII*, *NotI*, *XhoI*, *KpnI* and *XbaI*, and the SV40 small t intron and polyadenylation site.

2. *UAS-lacZ*: an *Adh-lacZ* fusion gene was removed from pCaSpeR-AUG-*-gal* (Thummel et al., 1988) by digestion with *EcoRI* and *XbaI*, and was subcloned in pUAST.

3. *UAS-even-skipped*: the *even-skipped* gene was subcloned as a *HincII* fragment from plasmid pE I (a gift from R. Warrior and M. Levine) into the plasmid pF91 that had been cut with *NotI* and made blunt using T4 polymerase, to give plasmid pF100. The *HincII* fragment contains the 5' end of the *even-skipped* cDNA (from an *XhoI* site to a *PstI* site) fused to the 3' end of the genomic *even-skipped* sequence (from the *PstI* site to an *EcoRI* site; MacDonald et al., 1986; Frasch et al., 1987). The *even-skipped* gene was then excised from pF100 as a *SmaI-XbaI* fragment and subcloned into pUAST that had been digested with *NotI* and *XbaI*, and the *NotI* site made blunt with T4 polymerase.

4. *UAS-Dras2<sup>Val14</sup>*: the *Dras2<sup>Val14</sup>* gene was excised from plasmid pBI20 7.7 *XhoI*#6 (a gift from J. Bishop and V. Corces) as a *HpaI-XhoI* fragment and was subcloned into plasmid pF91 that

had been digested with *KpnI* and *XhoI*, and the *KpnI* site made blunt with T4 polymerase.

### Injections

Transgenic lines were generated by injection of CsCl banded DNA, at a concentration of 600 µg/ml, into embryos of strain *y w; +/+; Sb, P[ry+, 2-3]/TM6, Ubx* (Robertson et al., 1988) using standard procedures (Santamaria, 1986; Spradling, 1986).

### Enhancer detection screen

Flies were raised on standard *Drosophila* medium at 25°C. Descriptions of balancers and mutations that are not described in the text can be found in Lindsley and Zimm (1992).

An enhancer detection screen was carried out to recover lines that express GAL4 in a cell- or tissue-specific manner. Insertion lines were generated by mobilizing a single X-linked enhancer detection GAL4 vector, pGawB. The first insertion line we used is hemizygous lethal and is carried over an *FM7* balancer chromosome (*GAL4-lethal/FM7*). We also mobilized a pGawB insertion from the 2.3, *Sb* chromosome. The P-element transposons were mobilized using the 'jumpstarter' strain *P[ry+; 2-3]*, which carries a defective P-element on the third chromosome at 99B (Laski et al., 1986; Robertson et al., 1988; Cooley et al., 1988). This P-element expresses high levels of a constitutively active transposase, but cannot itself transpose. The frequency with which new pGawB insertion lines were recovered was much lower than that previously reported for a similarly sized enhancer detection/*lacZ* P-element (N. P., unpublished data). This might be attributed to the alterations made in the sequence of the 5' end of the P-element pGawB that allow GAL4 to be expressed from its own AUG, rather than as a P-transposase-GAL4 fusion protein.

Insertions segregating with the X chromosome were detected by examining the segregation of *w+*. Autosomal insertions were mapped by standard genetic methods using the two stocks: *w; +/+; TM3, Sb/CxD* and *w; CyO/Sco; +/+*.

Each of the 220 lines was crossed to a line carrying either the *17-hsp70/lacZ* (Fischer et al., 1988) or the *UAS-lacZ* reporter gene. Embryos from the cross were collected on agar/molasses plates and stained for *-galactosidase* expression with anti-*-galactosidase* antibodies. A subset of the lines were also examined for *-galactosidase* expression in imaginal discs by staining with X-Gal.

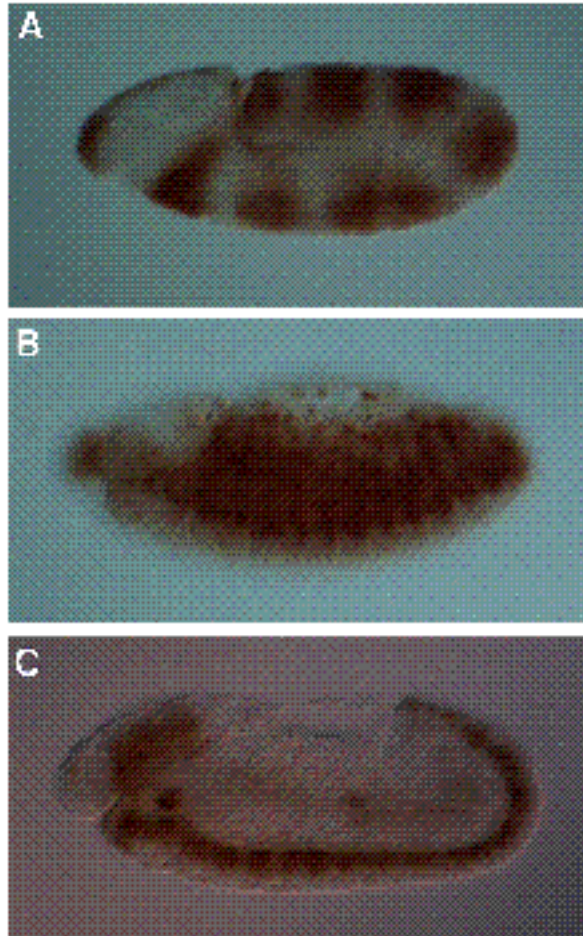
### Antibody immunocytochemistry and X-Gal staining

Embryos were dechorionated in 50% Clorox, fixed for 10 minutes in a 1:1 mix of heptane and 3.7% formaldehyde in PBS, 0.1% Triton X-100, and the vitelline membranes were removed by shaking in heptane/methanol. The embryos were incubated overnight at 4°C in a 1:1000 dilution of rabbit anti-*-galactosidase* antibody (a gift from B. Holmgren), or a 1:1 dilution of mouse anti-*even-skipped* antibody (a gift from N. Patel). After washing in PBS, 0.1% Triton X-100, embryos were incubated for 2 hours in biotinylated horse anti-mouse antibody (Vector Laboratories) at a 1:500 dilution, and then for 1 hour in avidin and biotinylated horseradish peroxidase (Vectastain Elite ABC kit, Vector Laboratories). Peroxidase was detected using diaminobenzidine (0.3 mg/ml in PBS, 0.1% Triton X-100) as a substrate. Embryos were then dehydrated in ethanol and mounted in methyl salicylate.

Imaginal discs were dissected in PBS, 0.1% Triton X-100 and fixed in 1% glutaraldehyde for 15 minutes. They were then stained in a solution of 10 mM Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> [pH 7.2], 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 3 mM K<sub>4</sub>[FeII(CN)<sub>6</sub>], 3 mM K<sub>3</sub>[FeIII(CN)<sub>6</sub>] containing a 1/50 dilution of X-Gal (25 mg/ml in dimethyl formamide). After washing in PBS, 0.1% Triton X-100, discs were mounted in 70% glycerol.

### In situ hybridization

Single stranded DNA probes were prepared as described by Patel

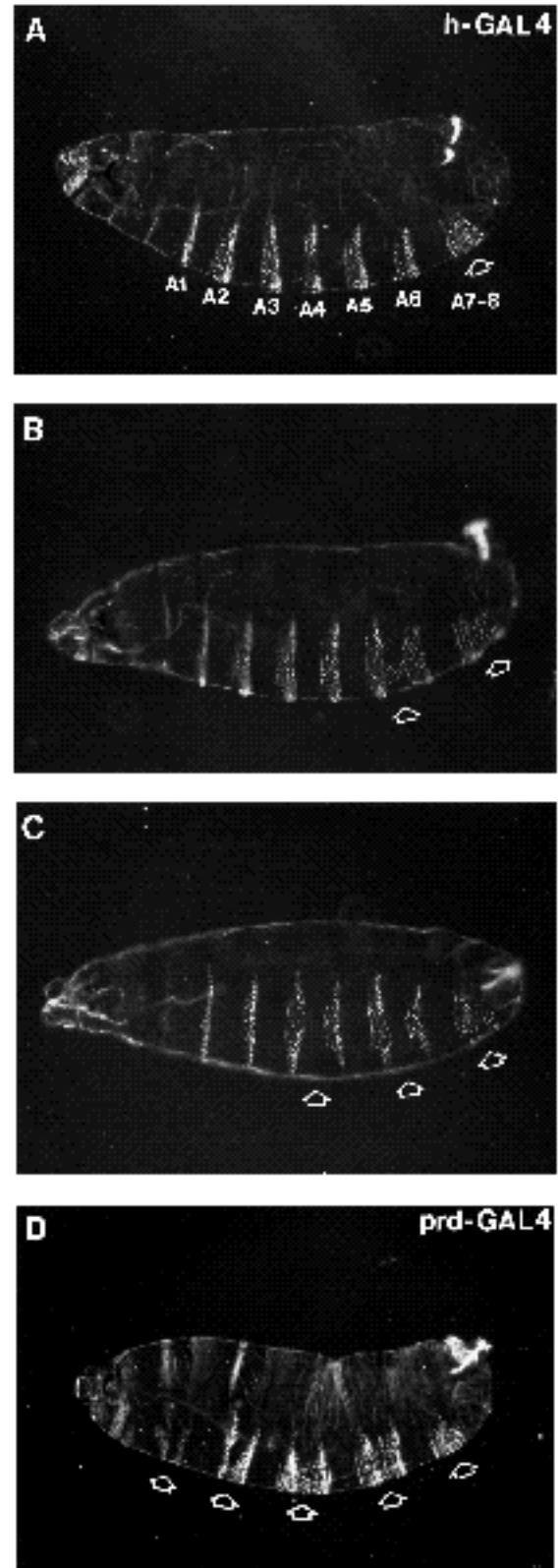


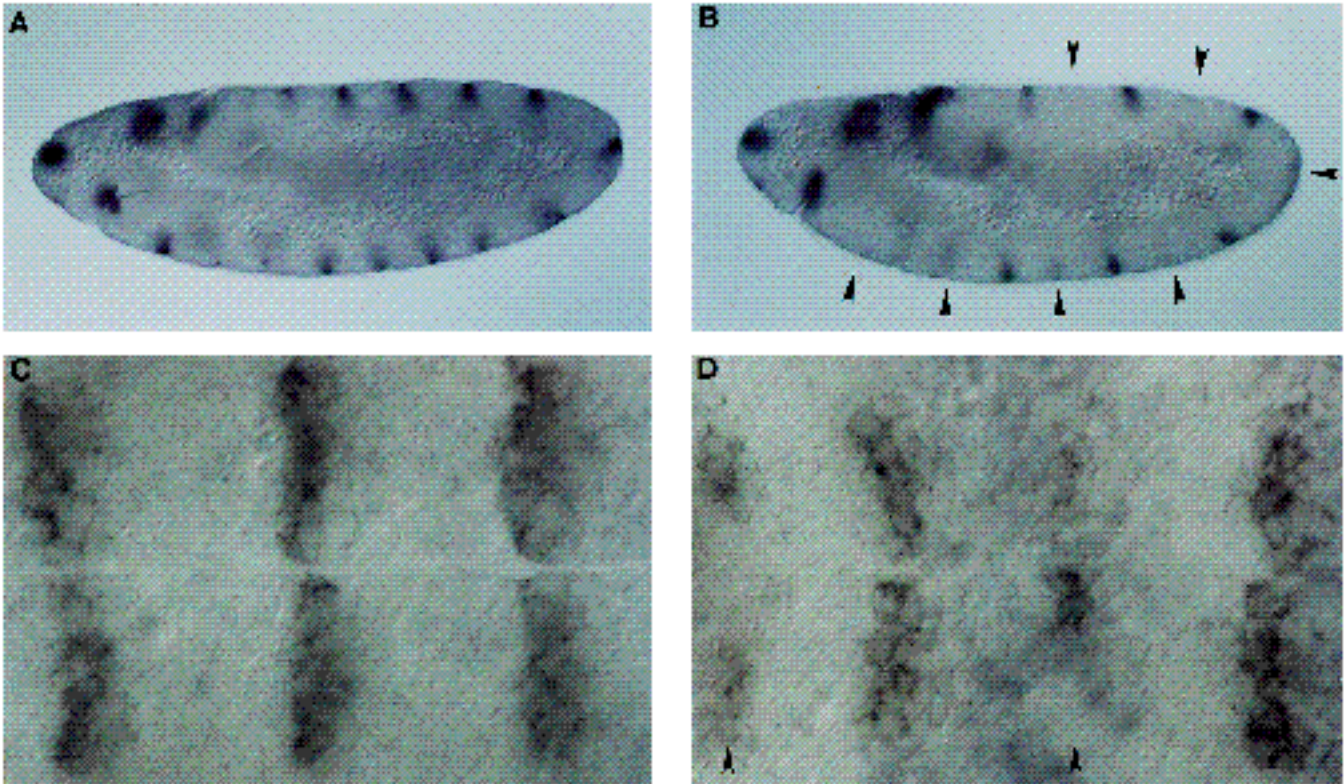
**Fig. 4.** GAL4-dependent misexpression of *even-skipped*. We used the same three GAL4-expressing lines shown in Fig. 3 to direct ectopic expression of the *even-skipped* gene. Lines 1J3, 24B and 31-1 were crossed to a *UAS-even-skipped* line (*eve-2-7-1*) and the progeny of the cross were stained with anti-*even-skipped* antibodies (3C10; N. Patel and C.S. Goodman, unpublished data). (A) Line 1J3 directs *even-skipped* expression in seven stripes and in the head of a stage 9 embryo (compare the ectopic *even-skipped* expression shown here with the  $\beta$ -galactosidase expression in Fig. 3A). (B) Line 24B drives *even-skipped* expression in the muscles of a stage 13 embryo (compare with the  $\beta$ -galactosidase expression pattern in Fig. 3C). (C) Line 31-1 activates *UAS-even-skipped* in cells throughout the central nervous system of a stage 12 embryo (see Fig. 3F for the comparable  $\beta$ -galactosidase expression pattern). In all photographs anterior is to the left, ventral at the bottom.

and Goodman (1992). In situ hybridization to whole-mount embryos was carried out as described by Ephrussi et al. (1991), except that embryos were fixed in 3.7% formaldehyde, without DMSO. In addition, the 1 hour incubation in methanol/DMSO at  $-20^{\circ}\text{C}$  was omitted. Embryos were cleared in 50% glycerol for several hours and then mounted in 70% glycerol.

#### Cuticle preparations

Cuticles were prepared and mounted as described by Struhl (1989).





**Fig. 6.** Misexpression of *even-skipped* selectively represses *wingless* expression. The loss of *wingless* between 6 and 9.5 hours of development leads to the appearance of extra denticles (Bejsovec and Martinez Arias, 1991; Dougan and DiNardo, 1992). Since misexpression of *even-skipped* gives a similar phenotype, we assayed whether ectopic *even-skipped* represses *wingless* transcription. *wingless* transcription in (A) a wild-type embryo at stage 11 (5:20-7:20 hours AEL), as assayed by in situ hybridization, and in B an embryo in which *UAS-even-skipped* expression is driven by *paired-GAL4* (stage 10; 4:20-5:20 hours AEL). In B *wingless* transcription is repressed in alternating segments throughout the embryo (arrowheads). (C) A wild-type embryo (about stage 11) stained with anti-*wingless* and anti-*even-skipped* antibodies. At this stage of development *wingless*, but not *even-skipped*, is expressed in stripes. (D) An embryo (about stage 11) in which *UAS-even-skipped* expression is driven by *hairy-GAL4*, stained with anti-*wingless* and anti-*even-skipped* antibodies. The *wingless* expressing cells are brown and the *even-skipped* expressing cells are a very pale purple. *wingless* is repressed (arrowhead) in those cells that ectopically express *even-skipped*. The anti-*even-skipped* staining is underdeveloped so as not to obscure *wingless* staining. In all photographs, anterior is to the left; A and B are lateral views; C and D are dorsal views of germ band extended embryos.

**Fig. 5.** Ectopic expression of *even-skipped* leads to segmental fusions. The GAL4-expressing line 1J3 directs expression of *even-skipped* in a pattern corresponding to that of the *hairy* gene (Fig. 4A). Cuticles prepared from these embryos exhibit fusions in alternating pairs of segments. (A) An embryo in which abdominal segments seven and eight are fused (arrow). (B) An embryo in which abdominal segments five and six, then seven and eight are fused (arrows), and (C) an embryo in which abdominal segments three and four, five and six, and seven and eight are fused (arrows). These phenotypes were observed in 68 out of 103 embryos in one experiment. More extensive fusions (e.g. A3-A4 and A5-A8; A6-A8; A3-A8) were observed in 15 embryos, while the remainder appeared wild-type. To show that the varied expressivity is not a general property of GAL4-mediated activation, but is due rather to GAL4 expression from the *hairy* promoter, we used a *paired-GAL4* construct to drive expression of *UAS-even-skipped*. In the progeny of this cross, as shown in D, 75% of the embryos show alternating segmental fusions along the entire length of the cuticle (arrows indicate thoracic segments T2-3 and abdominal segments A1-A2, A3-A4, A5-A6 and A7-A8). In all photographs anterior is to the left.

## RESULTS

### GAL4 expressed from the *Rh2* promoter activates transcription in the photoreceptor cells of the ocelli

We constructed a vector into which promoters can be inserted upstream of the GAL4 coding sequence (pGaTB; Fig. 2A). Transcriptional regulatory sequences are subcloned into the unique *Bam*HI site to create a gene that includes the promoter of choice fused to the GAL4 coding sequence, which is in turn linked to the transcriptional termination sequence of the *hsp70* gene.

To test whether GAL4-activated transcription is both specific and efficient, we used the promoter of the *Rh2* gene to drive GAL4 expression. The *Rh2* promoter is active in the photoreceptor cells of the ocelli, which are three light sensing organs located on the top of the *Drosophila* head (Mismer et al., 1988; Pollock and Benzer, 1988). Transgenic lines carrying the *Rh2-GAL4* gene were crossed to a line that carries the *E. coli lacZ* gene regulated by GAL4 binding sites (*UAS-lacZ*; see below; Fig. 2B). In the progeny of this cross GAL4 should be expressed in the pho-

toreceptor cells of the ocelli, where it should activate transcription of the *UAS-lacZ* gene.

Fig. 2B shows the heads of two flies: on the left is a fly that carries both the *Rh2-GAL4* and the *UAS-lacZ* fusion genes and on the right, one that carries only the *UAS-lacZ* gene. In an assay for  $\beta$ -galactosidase expression, the ocelli of flies carrying both the *Rh2-GAL4* and the *UAS-lacZ* fusion genes stain dark blue after a 15 minute incubation with the chromogenic substrate X-Gal, whereas the ocelli of flies that carry only the *UAS-lacZ* gene do not stain.

### Enhancer detection to express GAL4 in cell- and tissue-specific patterns

To generate many patterns of GAL4 expression rapidly, without having to rely on the availability of suitable promoters, we constructed an enhancer detection vector in which GAL4 transcription is directed by the weak promoter of the P-transposase gene (pGawB; Fig. 2A). With this vector, the enhancerless GAL4 gene can be integrated at random in the genome, bringing it under the control of a diverse array of genomic enhancers. The previously described enhancer detection vectors express a P-transposase/ $\beta$ -galactosidase fusion protein that is directed to the nucleus by a nuclear localization signal within the P-transposase sequence (O'Kane and Gehring, 1987; Bier et al., 1989; Bellen et al., 1989; Wilson et al., 1989). Since the amino terminus of GAL4 directs both nuclear localization and DNA binding (Silver et al., 1984; Keegan et al., 1986), the enhancer trap/GAL4 vector, pGawB, was designed to express GAL4 from its own AUG, but is otherwise similar to the *lacZ* enhancer detection vector, plwB (Wilson et al., 1989; see Fig. 2A and Materials and methods).

To make target genes responsive to transcriptional activation by GAL4, we constructed a vector into which genes can be subcloned downstream of five tandemly arrayed, high affinity GAL4 binding sites and upstream of the SV40 transcriptional terminator (pUAST; Fig. 2A). To test pUAST, the *lacZ* gene was inserted within the polylinker sequence to create the GAL4-responsive gene, *UAS-lacZ* (Fig. 2B).

Transgenic lines carrying pGawB were generated by P-element transformation. To ensure that these transformants express GAL4, each line was crossed to flies carrying *UAS-lacZ*, and the resultant progeny were stained for  $\beta$ -galactosidase activity. A line with pGawB inserted within an essential gene on the X-chromosome (*GAL4-lethal*) was used as a starter line to mobilize pGawB to different sites in the genome by introduction of a constitutively active P-transposase gene (2-3; Laski et al., 1986; Robertson et al., 1988; Cooley et al., 1988; see Materials and methods).

We examined 220 independent insertion lines. To identify the cells in which GAL4 activates transcription, each insertion line was crossed to the *UAS-lacZ* line, and the progeny were stained with anti- $\beta$ -galactosidase antibodies. In this screen, 61% of the GAL4 insertions activate *UAS-lacZ* in a specific embryonic pattern. The distribution of patterns is shown in Table 1. In addition to activating expression in specific embryonic patterns, 51% of the insertions also activate transcription in the salivary glands and 28% activate in the salivary glands alone, suggesting that in constructing the GAL4 vector we may have generated a

**Table 1. Patterns of GAL4-directed expression in embryos**

No. of strains	No staining	Salivary glands	Other tissues	%
23	+	-	-	10.45
62	-	+	-	28.2
112	-	+	+	50.9
23	-	-	+	10.45
Total	220			100.00

position-dependent salivary gland enhancer. The earliest time in embryonic development that we have been able to detect GAL4-mediated expression is just after gastrulation, between 3.5 and 4 hours after fertilization.

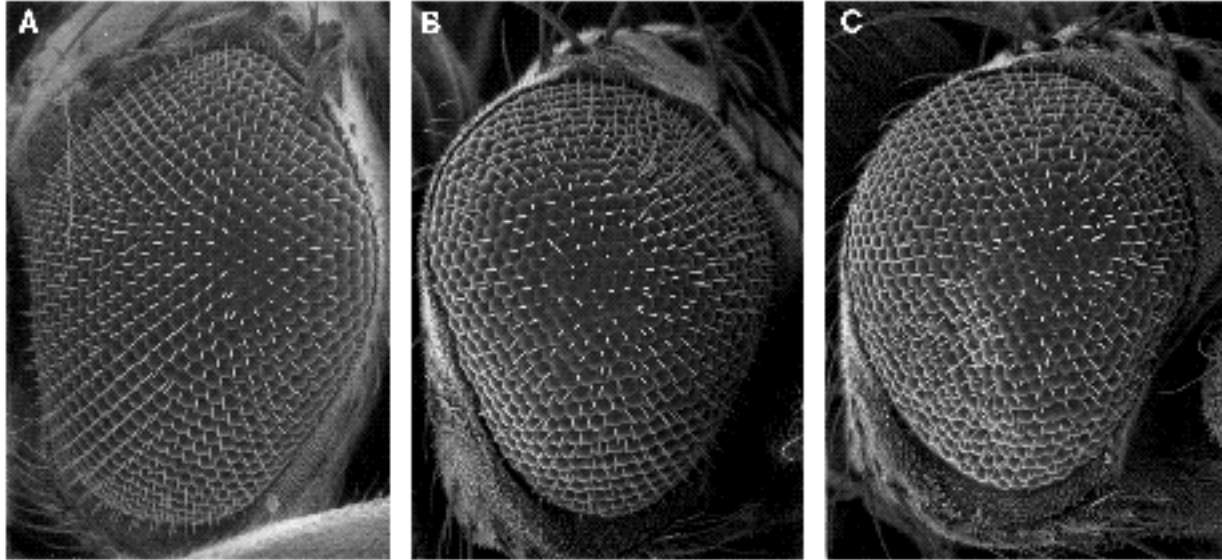
### GAL4 expression patterns

Many of the GAL4 lines we generated can be used to activate genes in specific embryonic tissues. In Fig. 3 we show a representative group of GAL4-expressing lines. The GAL4 insertion 1J3 activates transcription of *UAS-lacZ* in a pair-rule pattern of seven stripes, as assayed by staining with anti- $\beta$ -galactosidase antibodies (Fig. 3A). This expression pattern resembles that of the gene *hairy* with respect to both the number and placement of the stripes, and the pattern of expression in the head (Carroll et al., 1988; Hooper et al., 1989). In common with the *hairy* protein expression pattern, stripe four is fused to stripe three at gastrulation and is somewhat weaker than the other stripes. Ectodermal *hairy* expression begins to decay at gastrulation, while expression endures within the mesoderm until stage 9/10, at the end of germ band extension (Hooper et al., 1989). Ectodermal  $\beta$ -galactosidase expression directed by GAL4 insertion 1J3 persists through germ band extension, and eventually fades. Expression is maintained in the mesoderm and is later observed within muscle cells. A *lacZ* enhancer detection vector inserted at the *hairy* locus gives a  $\beta$ -galactosidase expression pattern similar to that directed by line 1J3. The prolonged period of expression in stripes might be due to increased mRNA stability, or to perdurance of  $\beta$ -galactosidase in the former case, and of the GAL4 protein and the target gene product in the latter.

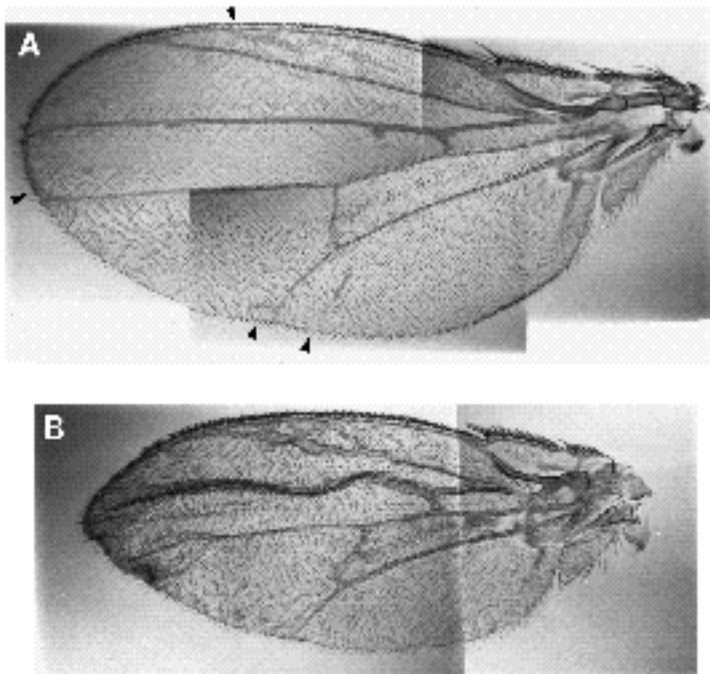
When the 1J3 insertion is made homozygous, viable adults exhibiting extra scutellar bristles are generated, a phenotype associated with several *hairy* alleles (Ingham et al., 1985). Adults that carry the 1J3 insertion in *trans* to *hairy* allele *h<sup>SHO7</sup>* also exhibit extra scutellar bristles, suggesting that in line 1J3 pGawB has inserted at the *hairy* locus.

Expression of GAL4 by insertion 24B causes *UAS-lacZ* to be transcribed first in the presumptive mesoderm, as observed at germ band extension (Fig. 3B), and then in muscle cells (Fig. 3C). Insertion 31-1 activates *UAS-lacZ* in neuronal precursor cells, or neuroblasts, at stage 11 (Fig. 3D,E) and then within neurons of both the central and peripheral nervous systems (Fig. 3F,G). Other embryonic tissues or cells in which GAL4 can activate *UAS-lacZ* include the amnioserosa, the foregut, midgut and hindgut, the pharynx, the malpighian tubules, vitellophages, oenocytes and glia.





**Fig. 7.** GAL4-dependent expression of *Dras2<sup>Val14</sup>* leads to a rough eye phenotype. Scanning electron micrographs of compound eyes from flies with the following genotypes: (A) wild type; (B) GAL4 insertion 32B driving expression of *UAS-Dras2<sup>Val14</sup>*, and (C) GAL4 insertion 69B driving expression of *UAS-Dras2<sup>Val14</sup>*, demonstrating the roughening caused by targeted expression of *Dras2<sup>Val14</sup>*.



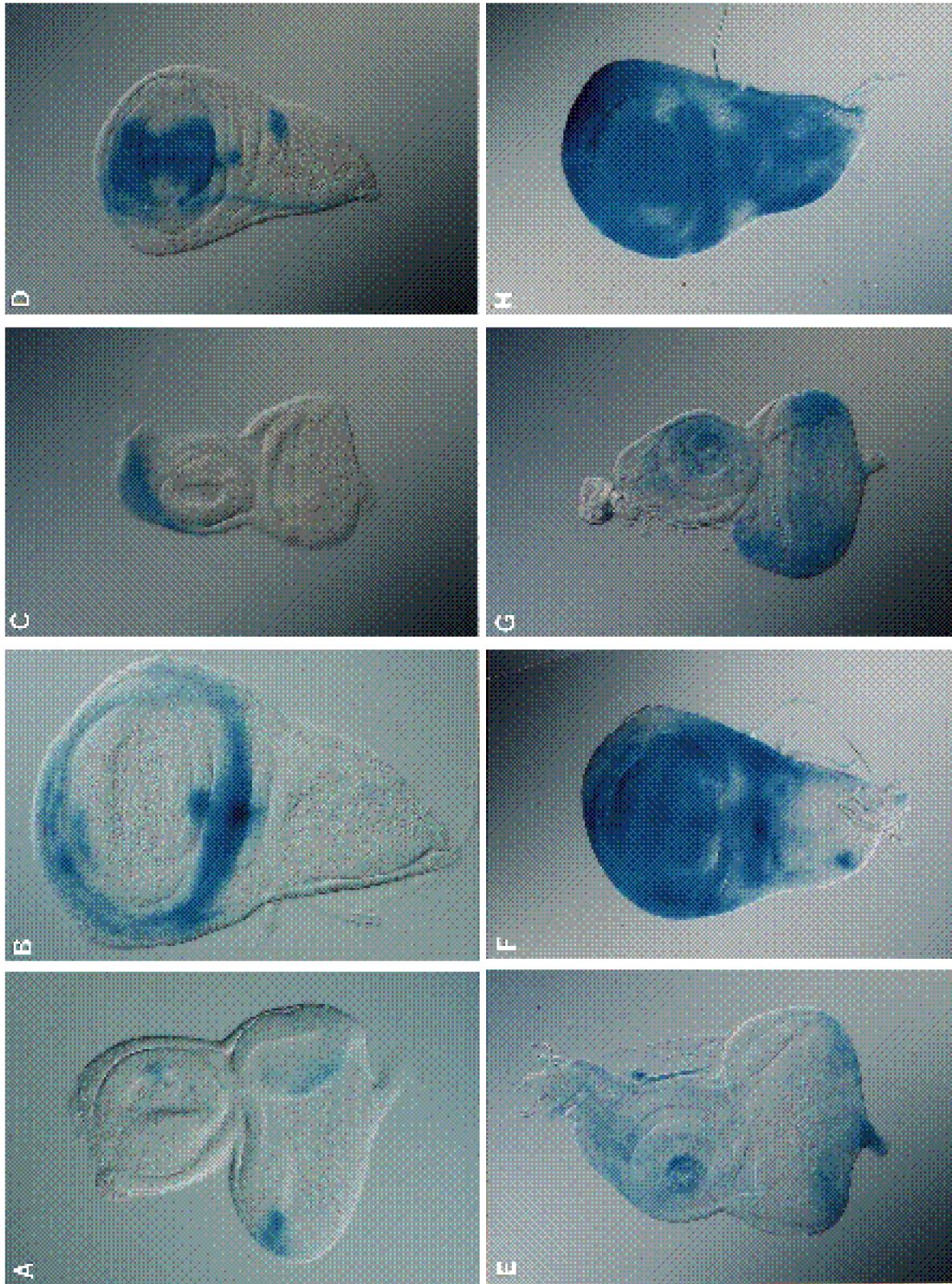
**Fig. 8.** Targeted expression of *Dras2<sup>Val14</sup>* disrupts wing development. (A) A wing from a fly in which GAL4 insertion 32B drives *UAS-Dras2<sup>Val14</sup>* expression: the longitudinal veins bifurcate at the wing margin (arrow), and extra vein material is evident (arrow). (B) A more severe phenotype is seen in flies where GAL4-insertion 69B drives *UAS-Dras2<sup>Val14</sup>* expression: the wing is reduced in size and exhibits broadened and disorganized veins. These flies rarely eclose.

### Altering cell fates by targeted expression of *even-skipped*

Targeted misexpression is a promising approach for examining the roles of different regulatory proteins in specifying cell fates. We used GAL4 to target expression of the *even-skipped* gene in an attempt to direct specific cell fate changes. *even-skipped* encodes a homeodomain protein that is involved in determining cell fate in the ectoderm, during embryonic segmentation, and later in the central nervous system (Frasch et al., 1988; Doe et al., 1988). *even-skipped* is expressed at the blastoderm stage in seven stripes, cor-

responding to the odd-numbered parasegments. By stage 7, at 3 hours of development, the stripes have faded and expression is not observed again until after germ band retraction when *even-skipped* can be detected in the central nervous system in about fourteen neurons per hemisegment (Patel et al., 1989).

To test if GAL4 can activate *UAS-even-skipped* to levels that result in embryonic phenotypes, transgenic lines carrying *UAS-even-skipped* were crossed to the GAL4-expressing lines shown in Fig. 3. The progeny of each cross were then stained with anti-*even-skipped* antibodies (Fig. 4). Fig. 4A shows GAL4-insertion IJ3 activating *even-*



**Fig. 9.** GAL4 expression in imaginal discs. Four GAL4-expressing lines that drive expression of *UAS-Dras2<sup>Val14</sup>* were crossed to a *UAS-lacZ* line ( $\beta$ -gal-1-2), and the progeny were allowed to develop to the third instar larval stage. The larvae were then dissected and their imaginal discs were stained with X-GAL to detect  $\beta$ -galactosidase activity. Shown here are the eye and wing imaginal discs from: (A,B) line 71B, which gives rise to a *UAS-Dras2<sup>Val14</sup>*-dependent wing phenotype; (C,D) line 30A, which gives a *UAS-Dras2<sup>Val14</sup>*-dependent wing phenotype; (E,F) line 32B, which gives rise to a *UAS-Dras2<sup>Val14</sup>*-dependent phenotype in both the eye and the wing (see Figs 7B, 8A); (G,H) line 69B, which also gives a *UAS-Dras2<sup>Val14</sup>*-dependent eye and wing phenotype (see Figs 7C, 8B). In all photographs anterior is up.

*skipped* in the pair rule stripes corresponding to the *hairy* expression pattern. By this stage of development (stage 9, 3:40-4:20 hours) *even-skipped* expression would normally have faded, but expression persists due to GAL4-directed transcription. Fig. 4B shows GAL4 insertion 24B directing *even-skipped* expression in embryonic muscle. In Fig. 4C, *UAS-even-skipped* is activated by GAL4 insertion 31-1, causing *even-skipped* to be expressed throughout the nervous system, rather than in a specific subset of neurons. In each of these crosses, misexpression of *even-skipped* results in embryonic lethality.

Restricting expression within the animal permits cell fates to be altered in a chosen tissue or cell-type. Using the GAL4-insertion line 1J3, we can misexpress *even-skipped* in the ectoderm in the *hairy* pattern (Fig. 4A). In wild-type embryos at the cellular blastoderm stage, *even-skipped* is expressed in the odd-numbered parasegments, partly overlapping the stripes of *hairy* expression. The anterior border of *hairy* expression lies one to two cells anterior to the border of *even-skipped* expression (Carroll et al., 1988; Hooper et al., 1989) and thus encompasses the *wingless*-expressing cells. Between 6 and 9.5 hours of development, *wingless* is required to instruct cells to secrete naked cuticle: the loss of *wingless* in this time window leads to the appearance of extra denticles (Bejsovec and Martinez Arias, 1991; Dougan and DiNardo, 1992). One function of *even-skipped* during segmentation is to repress *wingless* transcription (reviewed by Ingham and Martinez Arias, 1992). Misexpression of *even-skipped* within the *wingless*-expressing cells might therefore switch off *wingless* transcription and effect a cell fate change such that cells that would normally secrete naked cuticle instead produce denticles.

Using the *hairy-GAL4* insertion line (1J3) we have activated *UAS-even-skipped* so as to expand the stripes of *even-skipped* expression anteriorly, into the *wingless*-expression domain, and to prolong *even-skipped* expression beyond the time when endogenous *even-skipped* has faded. This pattern of expression results in embryonic lethality and cuticles prepared from the embryos exhibit extra denticles between alternating segments, as shown in Fig. 5. Ectopic denticles can be seen in the normally naked region between abdominal segments three and four, five and six, and seven and eight (Fig. 5A-C). The expressivity of this phenotype is variable with respect to the number of segmental fusions observed in each embryo, with fusions occurring primarily in the most posterior segments. We attribute this variability to GAL4 expression directed by the *hairy* promoter, since a *paired-GAL4* construct (L. Fasano and C. Desplan, unpublished) driving expression of *UAS-even-skipped* causes alternating segmental fusions along the entire length of the embryo (Fig. 5D).

By targeting *even-skipped* transcription to the *wingless*-expressing cells in alternating segments we are able to elicit a change in epidermal cell fate within the naked region of the embryo. Since *wingless* is required to instruct cells to secrete naked cuticle, this phenotype suggests that *wingless* is being repressed. We assayed *wingless* expression in embryos in which *even-skipped* is ectopically expressed (Fig. 6). When *UAS-even-skipped* is activated by *paired-GAL4*, *wingless* transcription is turned off in alternating segments along the entire length of the embryo (Fig. 6B).

Activating *UAS-even-skipped* with *hairy-GAL4* leads to the repression of alternate *wingless* stripes primarily in the posterior abdominal region of the embryo (Fig. 6D).

### Generation of dominant adult phenotypes by directed expression of activated *Dras2*

We have used the GAL4 activation system to target expression of a constitutively active form of the *Dras2* protein. Our goal was first to establish viable transformant lines carrying the *UAS-activated Dras2* fusion gene, and second to generate stable *Dras2*-dependent phenotypes to be used in genetic screens. One advantage of a two-part activation system (eg. Fischer et al., 1988; Khillan et al., 1988; Byrne and Ruddle, 1989; Ornitz et al., 1991) is the ability to study the consequences of expressing products that might be toxic to the animal (Kunes and Steller, 1991). Using the GAL4 system, genes encoding lethal products can be subcloned into pUAST, where they are silent until GAL4 is introduced. Lines bearing the *UAS-gene* fusion are thus viable until crossed to a GAL4-expressing line. Only the progeny of such a cross will exhibit phenotypes resulting from the activity of the gene. Next, transcription of the gene can be targeted to non-essential tissues, to permit the generation of viable adults with visible phenotypes.

With the intention of generating a constitutively activated form of *Dras2*, Bishop and Corces (1988) converted the glycine residue at position 14 to valine, to produce *Dras2<sup>Val14</sup>*. This mutation, by analogy with mutations at position 12 of mammalian Ras, is thought to activate the protein. Transcription of *Dras2<sup>Val14</sup>* from a heat-shock promoter during third instar larval development kills 60-80% of the population. Surviving flies show eye and wing defects. Transcription of *Dras2<sup>Val14</sup>* from the *Dras2* promoter results in variable eye and wing defects, suggesting that *Dras2* may normally function in both eye and wing development (Bishop and Corces, 1988). The flies also exhibit reduced viability and fertility. Fortini et al. (1992) expressed *Dras2<sup>Val14</sup>* in the developing eye from the *sevenless* promoter, also producing a rough eye phenotype. In contrast to *Dras1*, *Dras2* does not appear to act downstream of *sevenless*, but appears to function in a separate signal transduction pathway.

We were able to generate several independent transgenic lines carrying the *UAS-Dras2<sup>Val14</sup>* fusion gene. All of these lines are viable and show no visible phenotypes in the absence of GAL4, suggesting that the *UAS-Dras2<sup>Val14</sup>* gene is silent. A *UAS-Dras2<sup>Val14</sup>* transgenic line (R52-a) was then crossed to several GAL4-expressing lines (1J3, 24B, 69B, 32B, 30A and 71B). In two crosses the progeny arrest as pupae (1J3 and 24B; data not shown); in one, as pupae and young adults (69B; Figs 7C, 8B), and in three crosses adult phenotypes are apparent (30A, 71B and 32B; Figs 7B, 8A). This suggests that the phenotypes we observe are due to GAL4 expression in the imaginal discs. To confirm this, we assayed for GAL4 expression in the imaginal discs of four of the GAL4 insertion lines and compared the GAL4 expression patterns with the *Dras2<sup>Val14</sup>*-dependent phenotypes produced. Fig. 9 shows the GAL4-dependent -galactosidase expression pattern observed in the eye and wing imaginal discs of lines 71B, 30A, 32B and 69B.

In crosses where GAL4-insertions 71B and 30A activate

*UAS-Dras2<sup>Val14</sup>*, flies show a mild wing defect, such that the wings appear slightly opaque (data not shown). Both of these lines express GAL4 in the wing imaginal disc (Fig. 9B,D, respectively). No obvious eye defects are observed, as might be expected, given the restricted GAL4 expression pattern in the eye imaginal disc in these lines (Fig. 9A,C, respectively). Activating *UAS-Dras2<sup>Val14</sup>* with GAL4-insertions 32B and 69B results in flies with a more severe wing defect, as well as a rough eye phenotype (Figs 7, 8). The wings are not only opaque, but also exhibit vein defects. Lines 32B and 69B express GAL4 at high levels in both the wing and the eye imaginal discs (for line 32B, compare the eye disc in Fig. 9E with the eye in Fig. 7B, the wing disc in Fig. 9F with the wing in Fig. 8A; for line 69B, compare Figs 9G and 7C for the eye, and Figs 9H and 8B for the wing). The high level of *UAS-Dras2<sup>Val14</sup>* expression driven by GAL4-insertion 69B results most often in pupal lethality, whereas expression driven by 32B leads to viable adults in which the *Dras2<sup>Val14</sup>* phenotype is fully penetrant (in a sample of 100 flies, all showed the *Dras2<sup>Val14</sup>*-dependent eye and wing phenotype).

Using enhancer detection/GAL4 insertion lines we have observed *Dras2<sup>Val14</sup>*-dependent phenotypes in the wing alone, or in both the wing and the eye. We can also generate a rough eye phenotype, in the absence of a wing defect, by activating *UAS-Dras2<sup>Val14</sup>* in the developing eye using GAL4 expressed from the *sevenless* promoter (*sev*-*enless-GAL4*, K. Basler, unpublished; A. B. and N. P., unpublished data). Since these phenotypes are stable and reproducible, in contrast to those generated by expression of *Dras2<sup>Val14</sup>* from a heat shock promoter, genetic screens can now be carried out with the aim of identifying second-site mutations that affect other proteins in the *Dras2* signal transduction pathway.

## DISCUSSION

### The GAL4 activation system

We have developed a two-part system for targeting gene expression in *Drosophila* that allows transcription to be restricted to specific cells or tissues in embryos, larvae and adults. Using enhancer detection we have generated transgenic lines that express the yeast transcriptional activator GAL4 in numerous different patterns and at various stages of development. To activate a gene specifically in a particular cell or tissue, we constructed target genes in which transcription is driven by GAL4 binding sites. Transgenic flies carrying the target gene are then crossed to a GAL4-expressing line, producing progeny in which the target gene is transcribed in a specific GAL4-dependent pattern.

The GAL4 system can be used to express any gene of interest ectopically, including one that might be lethal to the organism. In the absence of GAL4 the toxic target gene is silent, and is only activated in progeny arising from a cross to a GAL4-expressing line. If a protein is required in a number of developmental processes, or acts at several times in development, its separate roles can be conveniently studied by restricting ectopic expression to specific cells or tissues, or to a particular stage of development.

GAL4 can direct expression in a wide range of embry-

onic patterns in *Drosophila*, and does not appear to be excluded from particular embryonic cells or tissues. We have observed expression in cells derived from all three germ layers: the endoderm, from which the midgut derives, the ectoderm, from which the nervous system and the epidermis arise, and the mesoderm. The one tissue where we have not been able to detect GAL4 mediated expression is the female germ line. In enhancer detection/*lacZ* screens, about one third of the insertion lines express  $\beta$ -galactosidase in the germ line (Fasano and Kerridge, 1988). In contrast, none of the enhancer detection/GAL4 lines we screened show appreciable expression in the germ line (A.B. and N.P., unpublished data; D. McKearin, personal communication). GAL4 translation might be selectively repressed in the germ line, or the GAL4 message may be selectively degraded. Alternatively, GAL4 may activate transcription in conjunction with another protein that is not present in germ cells.

The earliest that we are able to detect GAL4-directed expression in embryos, by staining with antibodies against either  $\beta$ -galactosidase or *even-skipped*, is after the completion of gastrulation, between 3.5 and 4 hours of development. A GAL4 insertion at the *hairy* locus can direct expression in a pattern that resembles *hairy*, but only from about stage 8 (3:10 to 3:40 hours; Campos-Ortega and Hartenstein, 1985), an hour or so after the onset of stripes of *hairy* protein expression. Whereas GAL4 mRNA can be detected at the cellular blastoderm stage (K. Staehling-Hampton and F. M. Hoffmann, personal communication) transcription of the target gene is not seen until three and a half to four hours of development. Two possible explanations for the delay in activation in early embryos are: (1) that the GAL4 mRNA is not translated during early embryonic development, or (2) that GAL4-mediated transcriptional activation requires a co-activator, which is itself not expressed until three to four hours after fertilization. These alternative explanations will be distinguishable when antibodies that recognize GAL4 in *Drosophila* embryos become available.

### Expression of *even-skipped* in *hairy* stripes alters epidermal cell fate

The segmentation gene *even-skipped* encodes a homeodomain protein that directs cell fates in the ectoderm and in the central nervous system (Frasch et al., 1987, 1988; Doe et al., 1988). Each of these roles can be studied independently using the GAL4 system to target *even-skipped* expression. We have misexpressed *even-skipped* in the *hairy* and *paired* expression patterns, expanding the *even-skipped* stripes anteriorly into the even-numbered parasegments, and repressing *wingless* in those cells that ectopically express *even-skipped*. This ectopic expression alters the fate of cells that would normally secrete naked cuticle, such that they now secrete denticles. Since *wingless* is required for the secretion of naked cuticle (Bejsovec and Martinez Arias, 1991; Dougan and DiNardo, 1992), it is possible that the ectopic denticles we observe are a direct result of *even-skipped* repressing *wingless* in the posterior cells of the even-numbered parasegments.

We found cuticle defects in the embryos that ectopically express *even-skipped*, but observed no associated central

nervous system defects. We have performed reciprocal experiments in which we have been able to disrupt CNS development without affecting the cuticle (A.B. and N.P., unpublished data). GAL4-dependent targeted expression can thus be used effectively to study the roles of *even-skipped* in either epidermal or central nervous system development.

### Directed expression of activated *Dras2* to generate adult phenotypes for genetic analysis

The GAL4 system can be used to restrict the expression of lethal products, and in so doing, to generate dominant adult phenotypes for use in genetic screens. We have targeted expression of an activated form of the *Drosophila* Ras homologue, *Dras2*. Using a randomly integrated GAL4 gene to drive transcription, we have been able to target expression of *Dras2<sup>Val14</sup>* to the eye and wing imaginal discs, and to generate flies exhibiting a rough eye phenotype and wing vein defects, but normal viability and fertility. These mutants can now be used to identify other proteins in the *Dras2* signal transduction pathway by isolating second-site mutations that enhance or suppress the *Dras2<sup>Val14</sup>* phenotype. Furthermore, possible epistatic relationships can be tested by combining the *Dras2<sup>Val14</sup>* mutations with mutations that affect eye or wing development, such as EGF receptor mutations (Baker and Rubin, 1989; Clifford and Schupbach, 1989).

### Future prospects

In conclusion, we have developed a two-part activation system that allows the restricted expression of any gene of interest. We have utilized a transcriptional activator that can activate transcription in yeast, in *Drosophila*, in plants and in mammalian cells, such that the enhancer detection/GAL4 system we describe might be extended for use in other organisms. For example, enhancer- or promoter-detection/*lacZ* constructs have been developed for use in mice (Allen et al., 1988; Gossler et al., 1989), and could be modified to express GAL4.

GAL4 can be used to misexpress regulatory proteins, or to target expression of mutated proteins, producing either gain of function or dominant negative phenotypes. Mutant proteins from other species might be expressed in *Drosophila* to generate phenotypes for use in genetic screens. As an example, we have expressed an activated form of the human *c-raf1* gene in flies, generating embryonic and adult phenotypes consistent with the known roles in development of the *Drosophila raf* protein kinase, as well as uncovering previously unidentified functions (A.B. and N.P., unpublished data).

The GAL4 system allows the expression of genes encoding toxic products, such as activated oncogenes. More recently we have used GAL4 to target expression of the A chain of diphtheria toxin, and have demonstrated GAL4-dependent cell killing (A.B., J. Haseloff, H. M. Goodman and N.P., unpublished data). It is now possible to kill any cell expressing GAL4 and to study the consequences of cell- and tissue-specific ablation on embryonic, larval or adult development. For example, targeted killing within the nervous system may result in reproducible behavioral

abnormalities, such that the role of particular cells in generating behavior can be analyzed.

Finally, other components of the GAL regulatory pathway, which has been so thoroughly analyzed in yeast, could be imported into flies to diversify further the GAL4 system. For example, a temperature sensitive allele of GAL4 (Matsumoto et al., 1978) has been described that, if expressed in flies, would allow ectopic expression to be restricted temporally as well as spatially. Alternatively, GAL4 could be used to drive expression of target genes encoding temperature sensitive proteins. To refine further the pattern of GAL4-dependent transcription, a negative regulator of GAL4, the GAL80 protein, might be introduced into *Drosophila* and expressed in a pattern that overlaps that of GAL4. A temperature sensitive allele of GAL80 has also been described (Matsumoto et al., 1978).

The level of GAL4 induced expression can be modulated by increasing or decreasing the number of GAL4 binding sites upstream of the target gene, or by using GAL4 derivatives, or activators fused to the GAL4 DNA binding domain, that are capable of activating transcription to different degrees (Ma and Ptashne, 1987a,b; Gill and Ptashne, 1987; Johnston and Dover, 1988). We have constructed an enhancer detection vector with GAL4 derivative 236 (Ma and Ptashne, 1987a), which is about half as active in yeast as full length GAL4, and have found it to activate transcription in flies (A.B. and N.P., unpublished data). While we have exclusively discussed the activation of target genes with GAL4, it may also be possible to repress the expression of endogenous genes by using GAL4 to drive the transcription of antisense RNAs. GAL4 can activate and maintain transcription at high levels, which might improve the efficiency of sense/antisense interactions.

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