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GAL4 Enhancer Trap Patterns During *Drosophila* Development

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To identify genes expressed in interesting spatial and temporal patterns during development, we mobilized transposons carrying the yeast transcriptional activator, GAL4, to create new insertions throughout the *Drosophila* genome. At these new sites, neighboring enhancers drive expression of GAL4 in a pattern similar to that of nearby genes (Brand and Perrimon, 1993). Since GAL4 binds to a UAS target sequence, flies containing a GAL4 transposon can activate transcription of a UAS-linked gene, thereby resulting in controlled expression of that secondary gene. Thus, the GAL4 *P* element is a useful tool both for identifying genes of interest and for ectopically expressing genes in novel tissues or at specific developmental stages.

We mobilized a GAL4 insertion on the second chromosome (*P{GawB} CY2*; Queenan *et al.*, 1997) to generate a collection of GAL4-expressing enhancer trap lines. F1 "jumpstarter" males (w^{1118}*/Y; GAL4/CyO; Sb* Δ*2-3/+*) were mated in vials to attached-X (*C(1)DX/Y*) females. From 200 such crosses, red-eyed, curly-winged, long-bristled sons (bearing a new GAL4 insertion) were selected and mated individually to w^{1118}/w^{1118} females to establish 50 lines. Chromosome-mapping data, enhancer trap pattern, and other phenotypic characters demonstrated that four insertions were duplicates, yielding a total of 46 independent lines (Table 1). All X and third chromosome lines are viable, although three lines exhibit reduced viability or fertility (lines 15, 45, and 50). We could not determine the viability of the CyO-linked lines since the balancer chromosome is homozygous lethal. The genotypes of the resulting stocks are: X-chromosome lines, w^{1118} *P{GawB}*, second-chromosome lines, *y w; Pin/CyO, P{GawB}*, and third chromosome lines, *y w; III P{GawB}*. Line 15 also carries the *FM6, y w Bar* balancer while lines 45 and 50 contain *TM3, Sb Ser*. Finally, line number three exhibits a striking eye phenotype consisting of a variable loss of eye pigment in a gradient along the anterior/posterior axis. Surprisingly, this line lacks detectable GAL4 expression in larval eye discs.

We characterized sequences flanking a subset of the insertions in our collection by the inverse PCR method (http:www.fruitfly.org/methods/). Table 1 lists neighboring predicted or known genes.

To identify and visualize the morphology of cells expressing GAL4, we crossed females from each line to males carrying *UAS-taulacZ*, which links the β-galactosidase enzyme to the tubulin-binding protein Tau (Hildago *et al.*, 1995). Some lines (1, 7, 10 (males only), 14, 22, 25, 26, 32, 35, 36, and 41) were lethal in pupal stages when crossed to this reporter; therefore, to assay ovary and testis expression, we crossed these strains to *UAS-lacZ.NZ,* which contains a nuclear-localization signal (Y. Hiromi and S. West, unpubl. results).

Tables 1 and 2 describe the expression patterns for each line and Figure 1 shows selected patterns that illustrate key points. More detailed descriptions of all the patterns are available upon request. All lines exhibit interesting staining in at least one developmental stage. As commonly noted, we observe patchy or variable GAL4 expression in many lines. For example, GAL4 drives embryonic PNS expression in 18 lines (Table 1). While all embryos within a given strain exhibit a general PNS pattern that is consistent, within an individual embryo, slight variations often occur between segmental repeats (e.g., brackets in Fig. 1a).

Only a few lines express GAL4 in a single tissue at a specific developmental stage. For example, line 20 drives expression in the longitudinal visceral mesoderm from stage 12 onwards (Fig. 1c). When crossed to *UAS-GFP*, line 20 can be used to visualize this highly migratory tissue in vivo. As Tables 1 and 2 indicate, many lines express GAL4 in identical tissues. Nevertheless, important differences may exist in the distribution of expression within each tissue. For example, Fig. 1f–i shows representative wing discs from four lines that express GAL4 in all imaginal discs, the brain, and the central nervous system (IGD, brain, and CNS). The distinct patterns shown in the wing discs presumably reveal specific regulatory elements that govern expression of nearby

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Table 1
GAL4 Expression Patterns in Embryos and Larval Discs

Strain	Chromosome	Gene	Embryo	Larval Disc
cb01*	14C4	CG4411	EP; AS	IGD; BR; CNS
cb03	X		few; random	CNS
cb04	X		none	BR; CNS
cb05	19C	CG1631	CNS; AMX	LD; EAD; BR; CNS
cb06	X		CNS; PNS	BR(wk); CNS(wk)
cb07	8C7–8D10	Clot6012	EP; SM; CNS	IGD; BR; CNS
cb08	19E6–19E7	mgst1	SM; PNS; AMX	IGD(wk); BR(wk); CNS
cb09	X		PNS	BR; CNS
cb10	2D2–2D4	CG3835	EP; CNS(wk); LG	IGD(wk); BR; CNS
cb11	X		AMX	EAD; BR; CNS
cb12	X		none	BR; CNS
cb13	X		SM(wk); CNS; PNS	IGD; BR; CNS
cb14	7E2–7E3	CG11190	MG; CNS; PNS	WD, LD(wk); BR; CNS
cb15	X		SM	ND
cb16	50B8–50B9	CG6191	EP, SM, CNS, PNS	IGD; BR(wk); CNS
cb17	12B8–12B9	rdgB	none	BR; CNS
cb18	13D2–13D4	CG6340	none	none
cb19	2		FG(wk); HG(wk)	EAD; BR(wk); CNS(wk)
cb20	28A5–28A6	CG13791	Longitudinal VM	BR; CNS; MB
cb21	2		MG; PNS	BR; CNS
cb22	2		SM(wk); PNS	IGD; BR; CNS—all tissue?
cb23	2		few; random	IGD; BR; CNS
cb24	2		EP; CNS(wk); LG	IGD; CNS
cb25	2		ND	IGD; BR; CNS—all tissue?
cb26	2		CNS; PNS; AMX; BR	IGD; BR; CNS
cb27	2		few; random	IGD; BR; CNS
cb28	2		few; random	IGD; BR; CNS
cb29	2		none	BR; CNS
cb30	2		MG	LD; BR; CNS
cb31	2		none	ND
cb32*	2		Subset most tissues	BR; CNS
cb34	2		few, random	WD
cb35	2		ND	WD; BR; CNS
cb36	2		CNS; PNS	IGD; BR; CNS
cb37	2		FG; PNS; CLP	IGD(wk); BR; CNS
cb38	2		SM(wk); CNS; PNS; AMX	IGD; BR; CNS
cb39	2		ND	BR; CNS
cb40	2		PNS	none
cb41*	3		EP(P. Stripes); PNS; AMX	IGD; CNS
cb43*	3		CNS; PNS; BR	BR; CNS
cb45	3		PNS; AMX	LD; BR; CNS
cb46	3		few, CNS	BR; CNS
cb47	3		few, random	BR; CNS
cb48	3		SM(wk); PNS; AMX	IGD(wk); BR; CNS
cb49	3		SM(wk); PNS; AS	none
cb50	3		EP	IGD; BR(wk); CNS(wk)

From left to right: the strain number of each GAL4 line, the chromosome on which the *P* element is inserted, the closest predicted or known gene, and the embryonic and larval expression patterns. For duplicated lines (*), we retained only the first line. To describe the expression patterns we use the nomenclature listed below. In the majority of fly lines we did not detect GAL4-mediated expression in embryos until stage 13/14. Embryonic expression pattern: AMX, antennomaxillary complex; AS, amnioserosa; BR, brain; CL, clypeolabrum; CNS, central nervous system; EC, ectoderm; FG, foregut; HG, hindgut; LG, lymph gland; MG, midgut; PNS, peripheral nervous system; SM, somatic musculature; VM, visceral musculature. Larval expression patterns: EAD, eye antennal disc; IGD, all imaginal discs; LD, leg disc; MB, mushroom bodies; WD, wing disc; (wk), weak staining.

genes; thus, each GAL4 line exhibits unique characteristics.

Temporal and spatial differences in expression also occur in the ovary. For example, four lines drive GAL4 in anterior and posterior follicle cells, yet differences in the precise patterns reveal subpopulations of follicle cells. Line seven expresses GAL4 in the anteriormost and posteriormost follicle cells, including the border cells (Fig. 1k). In line six, the border cells lack expression in stage 9–11 egg chambers, but two border cells induce expression at the time of micropyle formation; posterior expression is variable (Fig. 1l). Line 14 expresses GAL4 uniformly in all stretch, centripetal, and posteriormost follicle cells (Fig. 1m), while line 24 exhibits expression in stretch cells, a subset of dorsal anterior cells, and strongly but patchily in the posterior (Fig. 1n). These differences provide tools for investigating enhancer functions and reveal the influence of chromatin struc-

Table 2
GAL4 Expression Patterns in the Ovary and Testis

Strain	Ovary	Testis	*UAS-ttk*
cb01*	TF+I; few FC S1–14; OoS8–13; DA; Aero	S	L/L
cb03	TF; FC S1–14 (wk); Oo S10 (wk); DA	M	V/V
cb04	TF; FC, NC, Oo S1–14 (wk)	W	L/V
cb05	region II + FC/Oo S10–13 (wk); NC, Oo S8–10; DA; Aero	N	18/V
cb06	few FC S9–14; MP S12–14; Aero	S	L/L
cb07	TF; FC germarium; ant/post FC; BC; Stalk	S	L/V
cb08	TF; FC, NC, Oo S8–13 (wk); FC S14	M	L/L
cb09	FC/Oo S8–14 (wk); Oo S10 (wk); DA	W	L/V
cb10	TF; FC/Oo S10–14 (wk); Oo S10 (wk); DA; Aero	ND	L/V
cb11	DA (wk)	N	V/V
cb12	TF; DA, Aero (wk)	W	M/V
cb13	All FC region II-S14; All germ cells region I-S14	R	L/L
cb14	few FC TF-S14; Stalk; ant/post FC S9–14	S	ND
cb15	few FC TF-S6; all FC S7–14	N	L/V
cb16	All FC TF-S14 (strong)	S	ND
cb17	TF; DA	S	V/V
cb18	TF; DA (wk)	S	ND/V
cb19	TF; FC S9–13 (wk); NC, Oo S6–14 (wk)	M	M/M
cb20	TF; NC near Oo S1–10 (wk); DA (wk)	S	L/V
cb21	TF; NC S1–7 (wk); DA	W	L/ND
cb22	NC, Oo S1–14	ND	L/L
cb23	TF; few FC S11–13; NC, Oo S6–14 (wk)	S	18/V
cb24	TF; BC, few FC S9; SC, CEN S10–14	R	L/ND
cb25	TF; NC S6 (wk); Oo (wk), post FC S8–14; few FC S10–14	ND	V/ND
cb26	TF+I; NC, Oo, FC S8–13 (wk); DA	M	L/L
cb27	few post FC S9–14; Oo S10–14 (wk)	S	V/L
cb28	FC late S14	M	L/V
cb29	NC S1–6 (wk); Oo S10 (wk)	W	L/V
cb30	TF; NC S1–6 (wk); Oo S10–14 (wk)	W	L/L
cb31	All FC region II-S14 (wk); All germ cells region I-S14 (wk)	W	V/L
cb32*	NC S1–10 (wk)	ND	L/L
cb34	FC S1–S6 (wk); FC/Oo S9–14 (wk); Oo S10–14	M	V/V
cb35	TF; FC S9–14	M	M/L
cb36	TF; Stalk; few FC S1–8; FC S9–14; NC S10 (wk)	S	M/L
cb37	TF; Oo S10; DA	S	M/ND
cb38	TF; FC region II-S14; Oo S10–14 (wk)	S	M/ND
cb39	none	S	L/ND
cb40	TF; FC, Oo S10–14 (wk)	W	V/V
cb41*	few FC S1–14; CEN S9–14	ND	ND
cb43*	TF; FC/Oo S10–14 (wk)	W	ND
cb45	TF; few FC S9–14; DA	N	ND
cb46	TF; FC S1–10 (wk); NC, Oo S10–14 (wk); few FC S10–14	W	ND
cb47	TF; FC S1–7(wk); NC, Oo S10–14 (wk); post FC S9–14	W	ND
cb48	TF; few FC S9–14	S	ND
cb49	Germ cells germarium-S14 (wk)	R	ND
cb50	TF; few FC S1–14	R	ND

From left to right: the GAL4-collection strain number, ovary and testis expression patterns, and viability with *UAS-ttk69* and *UAS-ttk88*. For duplicated lines (*), we retained only the first line. To describe the expression patterns we use the nomenclature listed below. Ovary expression pattern: Aero, aeropyle; ant, anterior; BC, border cell; CEN, centripetally migrating cells; DA, stage-14 dorsal-appendage forming cells; FC, all follicle cells; few FC, few random follicle cells at the specified stages; FC/Oo, follicle cells over the oocyte; MP, micropyle; NC, nurse cell; Oo, oocyte; post, posterior; S, stage; Stalk, stalk cells; TF, terminal filament and/or germline stem cells; TF+I, terminal filament and region I of germarium; (wk), weak staining. Since both reporter constructs expressed *lacZ* in the seminal vesicle regardless of the presence of GAL4, we scored testis expression as positive when it occurred throughout the tissue: S, strong; M, moderate; W, weak; R, circumferential rings around testis, and N, no expression. To examine the effects of misexpressing the transcriptional repressors encoded by *tramtrack (ttk)*, we crossed the X- and second-chromosome GAL4 lines to *UAS-ttk69* and *UAS-ttk88.* Most progeny from these crosses died prior to adulthood. We show the lethality induced by ectopic Ttk69 first, followed by the results from misexpressing Ttk88 (Ttk69/Ttk88): V = viable; L = lethal; M = moderately viable; and 18 = viable at 18°C.

ture on the control of GAL4-UAS interactions (Ahmad and Henikoff, 2001).

We also observe quantitative differences in GAL4 expression. For example, lines 13, 16, and 31 express GAL4 in all follicle cells at all times, but the levels of expression differ significantly. In contrast, expression in the germ line is weak (with the exception of line 22), perhaps due to the poor response of the basal promoter or termination signals in the *UAST* reporters (Rørth, 1998).

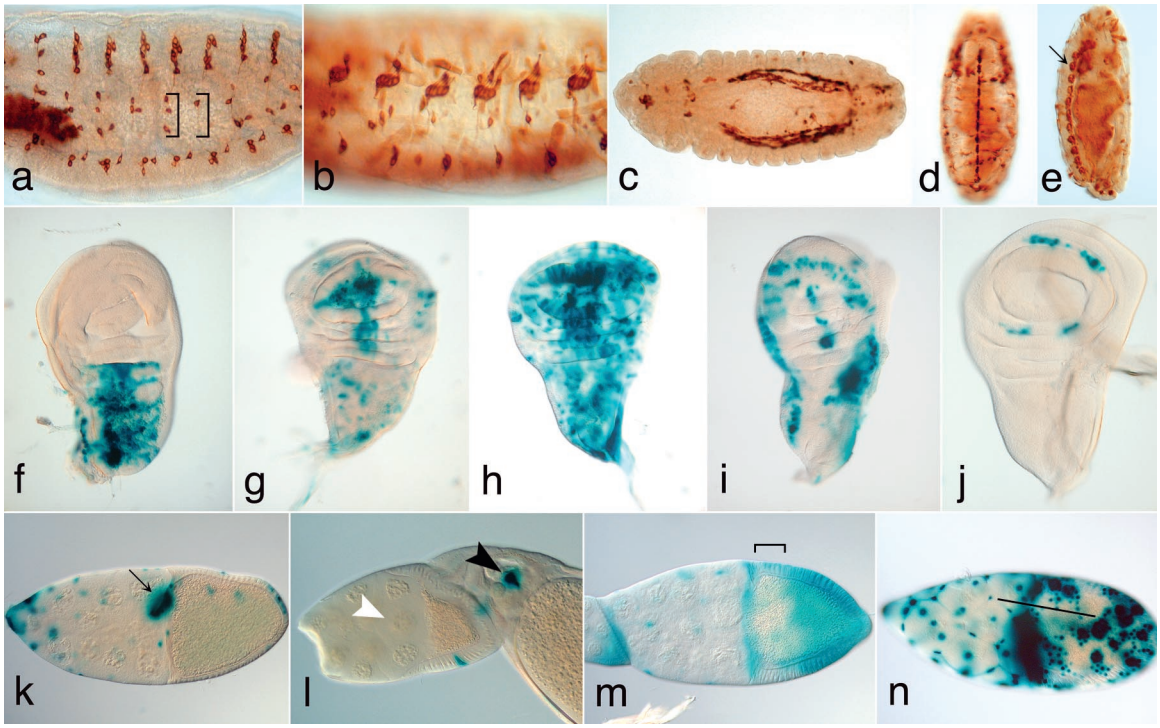

FIG. 1. Enhancer trap patterns in *Drosophila* tissues. We crossed the GAL4 enhancer-trap lines to flies carrying a *UAS-taulacZ* line to ascertain the spatiotemporal expression patterns of GAL4 and to reveal the morphology of the GAL4-expressing cells. We incubated fixed embryos with rabbit anti-β-galactosidase (1/3,000 Cappel, Malvern, PA) followed by biotinylated antirabbit secondary antibody (1/300, Vector, Burlingame, CA) and stained using the ABC and DAB Vector kits (Patel, 1994). We assayed fixed larval tissues, ovaries, and testes for β-galactosidase activity by using X-GAL (O'Kane, 1998). **a–e:** Selected embryonic expression patterns: (**a**) lateral view of sensory neurons in line 45; brackets reveal subtle differences in pattern between segments; (**b**) lateral view of chordotonal organs and weak somatic muscle expression in line 13; (**a,b**) (**c**) dorsal view of longitudinal visceral mesoderm expression in line 20; (**d**) ventral view, and (**e**) lateral view of the ventral nerve cord (arrow) showing strong dorsal midline expression in line 14. **f–j:** Selected wing disc expression patterns: (**f**) notum and dorsal hinge staining in line 13; (**g**) anterior/posterior and dorsal/ventral border staining in line 16; (**h**) expression in most wing disc cells in line 26; (**i**) ventral pleura and hinge staining in line 50; (**j**) sensilla campaniforma staining on the proximal ventral and dorsal radii in line 34. **k–n:** Selected ovary expression patterns: (**k**) anteriormost stretch cells, border cells (arrow), and a few posteriormost follicle cells in line 7; (**l**) in line 6, a few posterior cells stain, while border cells (white arrowhead) lack expression at stage 9; two micropyle cells (filled arrowhead) express *lacZ* beginning at stage 12; (**m**) line 14 shows expression in the anterior stretch and centripetal cells, the posterior two-thirds of follicle cells over the oocyte, while lateral cells do not stain (bracket); (**n**) line 24 shows expression in the stretch cells, two clusters of cells on either side of the dorsal, anterior midline (line), and random, posterior follicle cells.

By driving expression of rescue, gain-of-function (e.g., EP lines; Rørth, 1996) or dominant-negative constructs, GAL4 lines with different patterns may be used to test the precise spatial and temporal requirements for genes or pathways. We employed this approach to drive expression of *tramtrack* (*ttk*) (Giesen et al.; 1997), which encodes two different zinc-finger transcription factors (Read and Manley, 1992) with known functions in the nervous system. In many cases, ectopic expression of Ttk69 or Ttk88 during early development caused lethality (Table 2). Interestingly, some GAL4 lines induced lethality with one protein but not the other. This nonoverlapping distribution reveals functional differences in the activities of these two similar proteins.

In summary, GAL4 lines provide powerful tools for identifying genes, examining developmental events, and expressing genes of interest in defined patterns.

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