

# Generating Lineage-Specific Markers To Study *Drosophila* Development

NORBERT PERRIMON, ELIZABETH NOLL, KIMBERLY McCALL, AND ANDREA BRAND

Department of Genetics (N.P., E.N., K.M., A.B.) and Howard Hughes Medical Institute (N.P., E.N., K.M.), Harvard Medical School, Boston

**ABSTRACT** To generate cell- and tissue-specific expression patterns of the reporter gene *lacZ* in *Drosophila*, we have generated and characterized 1,426 independent insertion strains using four different P-element constructs. These four transposons carry a *lacZ* gene driven either by the weak promoter of the P-element transposase gene or by partial promoters from the *even-skipped*, *fushi-*tarazu**, or *engrailed* genes. The tissue-specific patterns of  $\beta$ -galactosidase expression that we are able to generate depend on the promoter utilized. We describe in detail 13 strains that can be used to follow specific cell lineages and demonstrate their utility in analyzing the phenotypes of developmental mutants. Insertion strains generated with P-elements that carry various sequences upstream of the *lacZ* gene exhibit an increased variety of expression patterns that can be used to study *Drosophila* development.

**Key words:** Enhancer detection, embryogenesis, cell lineage, P-element,  $\beta$ -galactosidase

specific patterns of expression, however, a large number of insertion strains must be examined, and many expression patterns are reiterated. We tested whether we could increase the frequency with which interesting patterns are recovered by mobilizing P-elements that contain different regulatory elements upstream of the *lacZ* gene. Since promoters and enhancers are often multipartite, and since patterns of transcription can be altered by either the addition or the removal of specific control sites, we speculated that the regulatory elements upstream of *lacZ* might interact with genomic promoters or enhancers to produce novel transcription patterns.

To test this possibility we compared the transcription patterns produced by insertions of the P[LArB] transposon with those generated by inserting a *lacZ* gene bearing various *cis*-acting control elements. Genomic enhancers may act in combination with these promoters to activate transcription. We find that both the type of patterns generated and the frequency with which tissue-specific patterns are recovered are altered by the presence of additional promoter elements upstream of the *lacZ* gene.

## INTRODUCTION

To express genes in specific tissues has required, until recently, the cloning and characterization of promoter regions. The enhancer detector method now allows regulatory elements to be detected *in situ*: A P-element that contains a *lacZ* gene driven by the weak promoter of the P-element transposase gene [i.e., the P[LArB] transposon; Wilson *et al.*, 1989] is mobilized, and its resultant transcription pattern is conferred by the site at which it integrates in the genome. The gene integrates randomly, often landing near a regulatory element that is able to direct tissue-specific expression of  $\beta$ -galactosidase [O'Kane and Gehring, 1987; Fasano and Kerridge, 1988; Bier *et al.*, 1989; Bellen *et al.*, 1989; Wilson *et al.*, 1989]. The enhancer detection technique, in combination with methods for mobilizing single P-elements [Cooley *et al.*, 1988; Robertson *et al.*, 1988], has allowed the production of numerous tissue-specific patterns of  $\beta$ -galactosidase expression that can be used to mark cells [Bier *et al.*, 1989; Bellen *et al.*, 1989]. To generate these highly

## MATERIALS AND METHODS

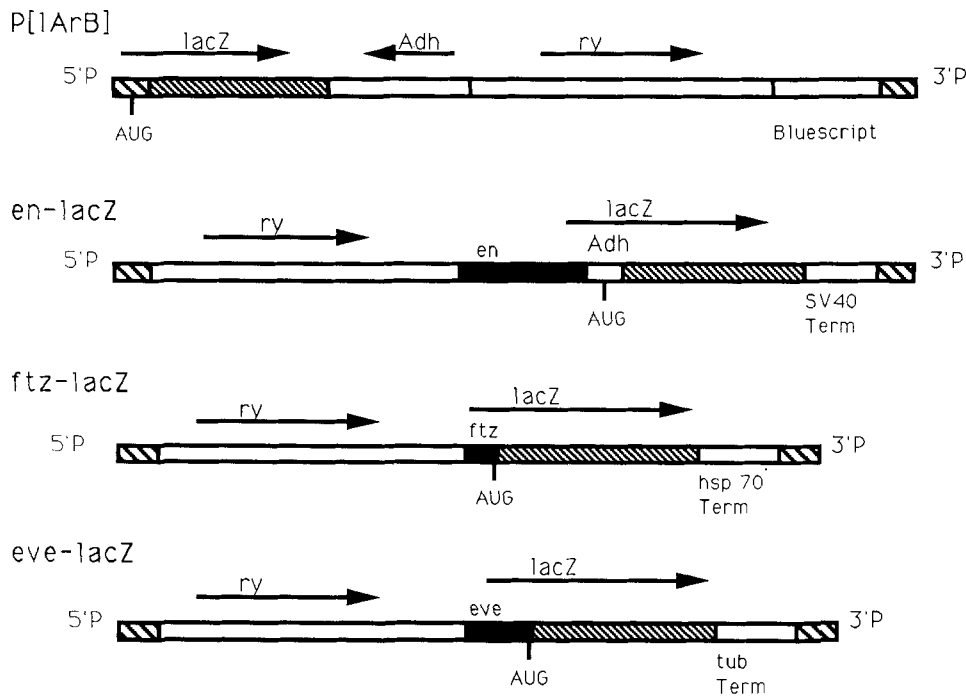
### P-Element Transposons

Four different transposons were mobilized. Schematic descriptions of these transposons are shown in Figure 1. The strains A22.1M2, A32.1M2, A80.1M2, and A66.2F2, which carry the P[LArB] transposon, were obtained from H. Bellen, U. Grossniklaus, and W. Gehring [Bellen *et al.*, 1989]. These strains carry a single P[LArB] transposon inserted at one of several locations on *CyO* balancer chromosomes. Since all four transposons exhibit similar rates of excision, the results of screens using these lines have been pooled.

The strain 5R13A (referred to as *en-lacZ*) was ob-

Received for publication November 6, 1990; accepted January 11, 1991.

Address reprint requests to Norbert Perrimon, Howard Hughes Medical Institute, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.



**Fig. 1.** The transposons. Four different transposons were used in this study, P[ArB], *eve-lacZ*, *ftz-lacZ*, and *en-lacZ*. The size of each element in the construct is approximate. See Results for references and details on the constructs. *Nomenclature: Adh*, alcohol dehydrogenase; *ry*, *rosy*; *en*, *engrailed*; *ftz*, *fushi-tarazu*; *eve*, *even skipped*.

Terminators from the *tubulin* (*tub term*), *hsp 70* (*hsp 70 term*), and *SV40* (*SV40 term*) genes. Note that, with the exception of the P[ArB] transposon, the promoter elements that control  $\beta$ -galactosidase expression are internal.

tained from J. Kassis [Kassis, 1990]. The *en-lacZ* strain has a single *en-lacZ* transposon inserted on the second chromosome and is homozygous viable.

The strain *5'Δ-239* (referred to as *ftz-lacZ*) was obtained from C. Dearolf and J. Topol [Dearolf *et al.*, 1989]. This strain carries a single *ftz-lacZ* transposon on the second chromosome and is homozygous viable.

The strain *sma 2* (referred to as *eve-lacZ*) was obtained from T. Goto [Goto *et al.*, 1989]. The *eve-lacZ* strain bears a single *eve-lacZ* transposon on the third chromosome and is homozygous viable.

Flies were raised on standard *Drosophila* media at 25°C. Descriptions of balancers and mutations that are not described in the text can be found in Lindsley and Grell [1968] and Lindsley and Zimm [1985, 1986, 1987].

### Genetic Scheme Used to Destabilize the P-Element Transposons

**Mobilization of the P[ArB] transposon (Fig. 2).** The mutator strain contains a unique transposon, P[ArB], marked with the *ry*<sup>+</sup> gene on the second chromosome balancer, *CyO*. The "jumpstarter" strain carries  $\Delta 2-3$ , a defective P-element located on the third chromosome at 99B, which constitutively expresses high levels of transposase but cannot itself transpose

[Robertson *et al.*, 1988].  $\Delta 2-3$  is marked with the *ry*<sup>+</sup> gene and is inserted on a chromosome carrying the dominant marker *Stubble* (*Sb*). For the F<sub>0</sub> cross, females that carry the mutator element are crossed en masse with males carrying the "jumpstarter" element. For the F<sub>1</sub> cross, males that carry both the mutator and "jumpstarter" elements are mass mated with *ry*<sup>-</sup> females in bottles. In the F<sub>2</sub> generation, both *ry*<sup>+</sup> males and *ry*<sup>+</sup> virgin females are isolated and single flies are used to establish a line by crossing with *ry*<sup>-</sup> flies. From a single bottle we usually established no more than 10–15 lines. Unbalanced insertion strains were stained for  $\beta$ -galactosidase expression. To eliminate identical transposition events derived from the same jumpstart male, only one insertion strain was kept when several lines originating from the same bottle demonstrated similar  $\beta$ -galactosidase expression patterns. Insertion strains with interesting staining patterns were subsequently balanced, and the established lines were stained again for  $\beta$ -galactosidase expression.

**Chromosomal segregations of the insertions.** Insertions that segregated with the X chromosome were readily detected by examining the segregation of *ry*<sup>+</sup> flies from the F<sub>2</sub> cross. Autosomal insertions were mapped following the cross of females heterozygous for the *ry*<sup>+</sup> marker to *CyO/+;MKRS,ry<sup>-</sup>/ry<sup>-</sup>* males. Subsequently, two *ry*<sup>+</sup> strains that carry either the *CyO* or

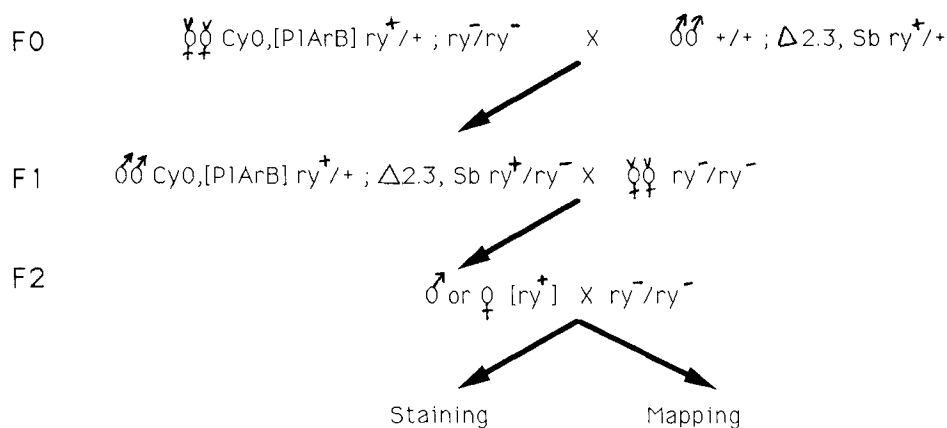


Fig. 2. Genetic scheme used to mobilize the *P[LA rB]* transposon. See Materials and Methods for details.

*MKRS* balancers were established. Progeny were examined for the presence of *ry*<sup>-</sup> flies.

**Mobilization of the other transposons.** Similar schemes were used to mobilize the transposons *ftz-lacZ*, *en-lacZ*, and *eve-lacZ*. The *en-lacZ* transposon was mobilized from an unmarked second chromosome, the *ftz-lacZ* transposon was mobilized from an unmarked second chromosome, and the *eve-lacZ* transposon, originally on the third chromosome, was first transposed onto a *Cy0* balancer, from which it was subsequently mobilized.

#### Cellular Localization of the $\beta$ -Galactosidase Protein

$\beta$ -Galactosidase encoded by the *P[LA rB]* transposon is localized in the nucleus, presumably because it is fused to the 128 N-terminal amino acids of the P-transposase protein [Bellen *et al.*, 1989].  $\beta$ -Galactosidase encoded by the three constructs *ftz-lacZ*, *eve-lacZ*, and *en-lacZ* is cytoplasmic.

#### Double Mutant Analysis

The expression of certain  $\beta$ -galactosidase-expressing strains was examined in various mutant backgrounds. The stocks used to generate the mutant embryos were *FM3/y fs(1)Nasrat<sup>211</sup> cho f; FM7c/y sc gt<sup>X11</sup> w<sup>a</sup>; CyO/sna<sup>II G05</sup>, cn bw sp; CyO/eve<sup>I.22</sup>, cn bw sp; Df(1)N8/In(1)dl49*.

#### Immunohistochemistry

$\beta$ -Galactosidase expression was detected either by enzymatic staining using X-gal as a substrate or by using a monoclonal antibody against  $\beta$ -galactosidase. X-gal staining was carried out as described by Ghysen and O'Kane [1989]. Embryos were prepared for immunohistochemistry using minor modifications of published procedures [Mitchison and Sedat, 1983]; histo-

chemical localization of horseradish peroxidase was performed as described [Ghysen *et al.*, 1986]. Briefly, embryos were dechorionated in 50% Clorox bleach, fixed for 2 min in phosphate-buffered saline (PBS)-buffered 4% paraformaldehyde/heptane, and devitellinized with absolute methanol. All steps were performed at room temperature. Following fixation, embryos were washed in PBS + 0.1% Triton X-100 (PT) and incubated overnight at 4°C with the anti- $\beta$ -galactosidase antibody (dilution 1:1,000; obtained from Promega). Embryos were then washed in PT for 2–4 hr at room temperature and incubated overnight at 4°C with biotinylated horse antimouse antibody (from Vector Laboratories) at a final concentration of 1:500. Embryos were washed in PT for 2–4 hr at room temperature. Visualization of horseradish peroxidase was performed using either a Vectastain peroxidase standard ABC kit or a Vectastain peroxidase Elite ABC kit. Labeled preparations were washed briefly, dehydrated in ethanol, cleared in methylsalicylate, and viewed using a Zeiss Axiophot microscope with Nomarski optics.

Embryonic patterns of  $\beta$ -galactosidase expression were detected by antibody, rather than X-gal, staining. The immunohistochemical detection of  $\beta$ -galactosidase is more reliable in our hands than X-gal staining, and the expression patterns are easier to analyze since there is no diffusion of the stain.

#### In Situ Hybridization to Polytene Chromosomes

In situ hybridization to polytene chromosomes was carried out using the chromosome squash procedure of Gall and Pardue [1971], with minor modifications, followed by hybridization with biotinylated probes [Langer-Safer *et al.*, 1982]. The probe used for in situ hybridizations, pDM23 [Mismer and Rubin, 1988] was random primed with biotinylated dUTP and detected using Detek-1-HRP from Enzo Diagnostic Inc.

TABLE 1. Summary of the Staining Patterns of Insertion Strains\*

	Transposon			
	p[LArB]	ftz-lacZ	eve-lacZ	en-lacZ
Number of strains	1,028	106	195	97
Staining patterns (%)	65	86	90	93
Specific staining (%)	16	15	17	65
Tissue specific (%)	4.8	11	7.7	41
Distribution of exclusive tissue-specific patterns				
Number of strains	49	12	15	40
Mesoderm	4	0	8	3
Endoderm	11	1	0	2
Ectoderm				
Epidermis	2	2	5	10
Neural (CNS and PNS)	30	9	2	25
Amnioserosa	1	0	0	0
Yolk	1	0	0	0

\*The number of strains stained for each transposon is indicated as well as the percentage of strains with detectable staining, specific staining, and tissue-specific staining. Strains with tissue-specific staining have been included with the strains that show specific staining. The strains with exclusive tissue-specific staining have been further classified according to the cell types stained.

## RESULTS

### The Screens

We designed screens (see Materials and Methods) to compare the expression patterns produced by transposition of the P[LArB] transposon [Wilson *et al.*, 1989], in which the *lacZ* gene is directly linked to the weak P-element promoter, with those generated by mobilizing the *lacZ* gene bearing various upstream *cis*-acting control elements. We suspected that transposition of a promoter fragment linked to the *lacZ* gene might alter the specificity of the  $\beta$ -galactosidase expression patterns obtained. First, we carried out an enhancer detection screen, similar to that described by Bellen *et al.* [1989], using the P[LArB] transposon. Second, we assayed the expression patterns generated by mobilizing transposons in which transcription of the *lacZ* gene is driven by promoter fragments from either the *engrailed* (*en*), *fushi tarazu* (*ftz*), or *even-skipped* (*eve*) genes. In these transposons, *lacZ* is located in approximately the middle of the P-element (Fig. 1). The P-elements bearing these different promoter regions are designated en-lacZ, ftz-lacZ, and eve-lacZ (see Materials and Methods and below).

For each transposon, we studied a number of strains and have classified those that show a staining pattern into three groups (Table 1): 1) those that show  $\beta$ -galactosidase expression, 2) those that have a specific staining pattern in a subset of embryonic cells (strains that express  $\beta$ -galactosidase ubiquitously or that express a basal pattern are excluded from this category), and 3) those that show exclusive tissue-specific expression of  $\beta$ -galactosidase (i.e., staining is observed in only one tissue type).

### P[LArB]

In the transposon P[LArB] (Fig. 1), the *lacZ* gene is driven by the weak promoter of the P-element trans-

posase gene. Integration near a genomic regulatory element drives transcription of the fusion gene. About 20% of the strains generated with the P[LArB] transposon give a basal pattern of expression similar to that seen for many different *lacZ*-containing P-element constructs [Bellen *et al.*, 1989; Ghysen and O'Kane, 1989]. The basal pattern of  $\beta$ -galactosidase expression occurs in stripes and is restricted to epidermal clusters of cells [Bienz *et al.*, 1988; Boulet and Scott, 1988; Kassis, 1990].

One thousand twenty-eight independent lines were generated by transposition of the P[LArB] transposon. The  $\beta$ -galactosidase expression patterns of all of these lines were examined, and at least 65% exhibited distinct  $\beta$ -galactosidase staining patterns in embryos (Table 1). Sixteen percent of the strains exhibited specific patterns of  $\beta$ -galactosidase expression, whereas the others exhibited either the basal pattern of transcription or ubiquitous expression. Transposition onto the X chromosome occurred in 7% of the lines. Three hundred twenty-six of the autosomal strains were mapped, and 152 (47%) segregated with the third chromosome, whereas 174 (53%) segregated with the second chromosome.

### ftz-lacZ

In this transposon, sequences extending from 239 nucleotides upstream of the *ftz* transcriptional start site to the second amino acid of the *ftz* coding region are fused to the *lacZ* gene (Fig. 1) [Dearolf *et al.*, 1989]. Within this *ftz* promoter region are found two repressor elements, R3 and R4, and two activator elements, A4 and A5, that affect the overall level of *ftz* transcription [see Dearolf *et al.*, 1989]. One hundred six independent lines were generated by transposition of the ftz-lacZ construct. The expression patterns of all lines were examined, and 86% show  $\beta$ -galactosidase expression in

embryos (Table 1). Fifteen percent of the strains show a specific pattern of expression, and the remaining strains that stain show the basal pattern of expression.

#### **eve-lacZ**

This transposon carries 1.65 kb of *eve* upstream regulatory sequences fused to the *lacZ* gene (Fig. 1) [Goto *et al.*, 1989]. This *eve* promoter region includes sequences sufficient for expression only of stripe 2 of the seven-stripped *even-skipped* pattern [see Goto *et al.*, 1989]. One hundred ninety-five independent lines were generated by transposition of *eve-lacZ*, and 90% show an embryonic  $\beta$ -galactosidase staining pattern (Table 1). Seventeen percent of the strains exhibit a specific pattern of expression; the others show the basal pattern of transcription in which a subset of the *eve* expression pattern is detectable [Goto *et al.*, 1989].

#### **en-lacZ**

The *en-lacZ* construct carries the 2.6 kb of the *en* promoter located immediately upstream of the *en-grailed* transcriptional start site (Fig. 1) [Kassis, 1990]. Although this sequence contains several homeodomain protein binding sites, it alone is insufficient to promote transcription of a fusion gene. If combined with the *en* promoter sequences normally located upstream of this fragment, the 2.6 kb element can direct transcription in stripes. Alternatively, regulatory sequences located in one of the *en* introns can substitute for the further upstream regulatory region [Kassis, 1990]. Ninety-seven independent lines were generated with the *en-lacZ* transposon, 93% of which showed  $\beta$ -galactosidase expression in embryos (Table 1). The recovery of strains carrying *en-lacZ* was much lower than previously reported for similar-sized P-elements. Although mobilization of the *en-lacZ* transposon was at least 20 times less frequent than the P[LArB] transposon, 65% of the strains demonstrate specific expression patterns. The remaining 35% show patterns of staining similar to the basal pattern exhibited by insertion strains carrying the P[LArB] transposon.

#### **Tissue Specificity of the Patterns**

Among the strains we characterized are several that can be used to mark every known embryonic tissue type, except the pole cells. The expression patterns of the strains were classified according to the germ layers stained. The classification is as follows (Table 1): 1) lines that express  $\beta$ -galactosidase in the mesoderm and its derivatives, i.e., muscle, heart, gonad; 2) lines that express  $\beta$ -galactosidase in the endoderm and the mid-gut derivatives; and 3) lines that express  $\beta$ -galactosidase in the ectoderm, subdivided into: 3a) the epidermis and its derivatives, such as foregut, hindgut, salivary glands, trachea, anal plates and spiracles, and 3b) staining in the nervous system (central nervous system [CNS], peripheral nervous system [PNS], and glial cells); 4) lines that express  $\beta$ -galactosidase in the

yolk nuclei, and, finally, 5) lines that express  $\beta$ -galactosidase in the amnioserosa.

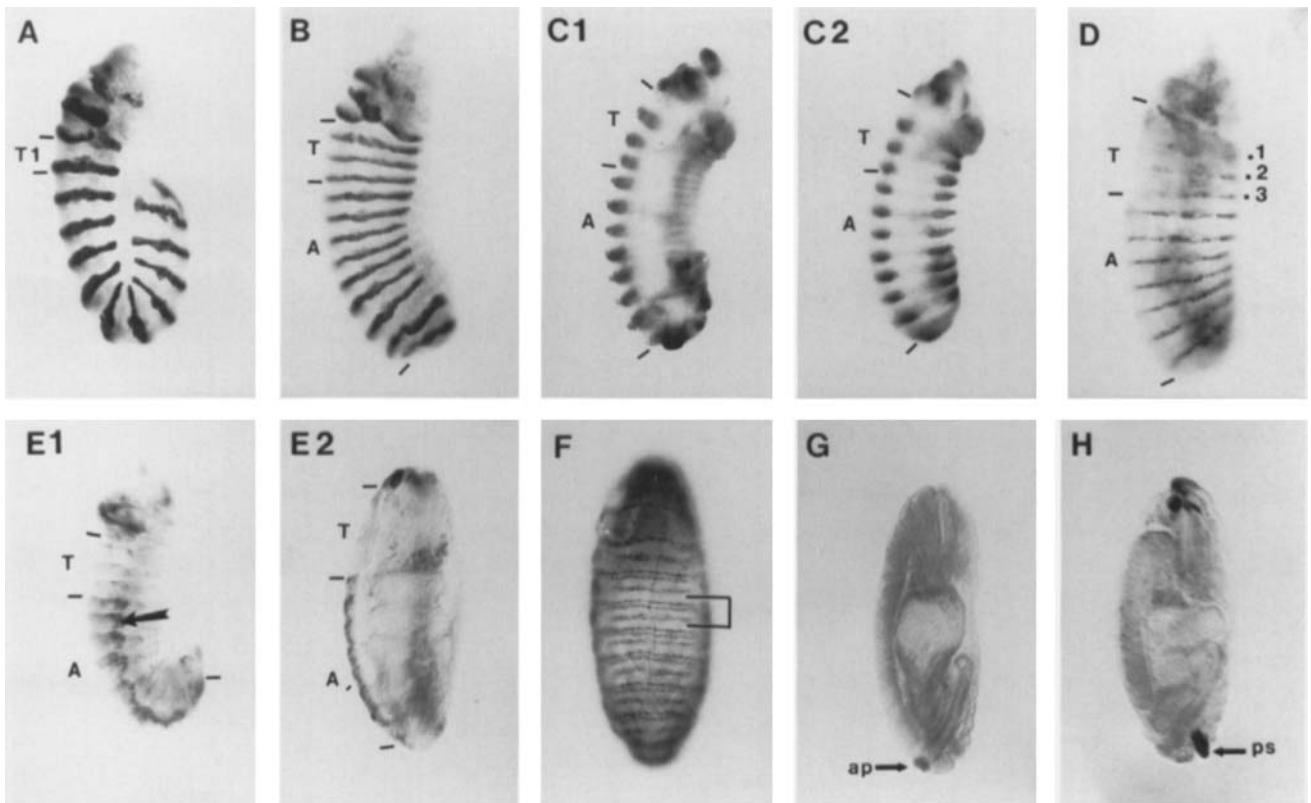
$\beta$ -Galactosidase expression that is restricted exclusively to one tissue is found in 4.8% of the P[LArB], 11% of the *ftz-lacZ*, 7.7% of the *eve-lacZ*, and 41% of the *en-lacZ* insertion strains (Table 1). Tissue specificity varies according to the transposon mobilized. For example, three of the constructs (P[LArB], *ftz-lacZ*, and *en-lacZ*) generate neural patterns at a much higher frequency than other patterns. In the case of the *eve-lacZ* transposon, mesodermal patterns are recovered preferentially. We also analyzed whether, in strains where two tissues stain, particular tissues always stain coincidentally. We selected 137 of the P[LArB] lines that show restricted expression patterns in only two tissue types. We could not detect any bias in staining of tissue types (data not shown).

As described above, the fraction of strains that produce tissue-specific patterns is quite similar for the three transposons P[LArB], *ftz-lacZ*, and *eve-lacZ* (Table 1). However, we find that most transpositions of the *en-lacZ* P-element yield tissue-specific expression patterns. Some of these patterns are shown in Figures 3 and 4. Many unique expression patterns are found in the epidermis and in the CNS (Figs. 3 and 4). Although 10% of the strains generated with this transposon express  $\beta$ -galactosidase in a segmentally repeated pattern that resembles the expression pattern of *en-grailed*, other patterns of expression in the epidermis and in the CNS are novel (Figs. 3C,E,F and 4).

#### **Strains That Allow Visualization of Specific Tissues**

We further characterized the strains that can be used as lineage-specific markers. To determine whether a particular  $\beta$ -galactosidase expression pattern follows a specific lineage, we examined all known progenitors of a specific anlagen and determined whether they stained for  $\beta$ -galactosidase. A strain was selected as a lineage marker if  $\beta$ -galactosidase expression labeled the tissue progenitors and all subsequent tissue derivatives. Using these criteria, we selected 13 strains (Table 2).

To demonstrate the use of these strains in studying embryonic development, we described the appearance of  $\beta$ -galactosidase during embryonic development in wild-type embryos as well as in various mutant backgrounds. Detailed descriptions of embryonic development can be found in Poulson [1950], Turner and Mahowald [1976, 1977, 1979], and Campos-Ortega and Hartenstein [1985]. Following fertilization, 13 rounds of nuclear divisions occur before cellularization, which culminates at 3 hr of development with the blastoderm stage. At this time, two kinds of nuclei are present, the cellularized diploid nuclei and polyploid vitellophages (or yolk nuclei) that are localized within the uncellularized yolk. Strain *IA115* specifically stains these yolk nuclei, which can be found later in development



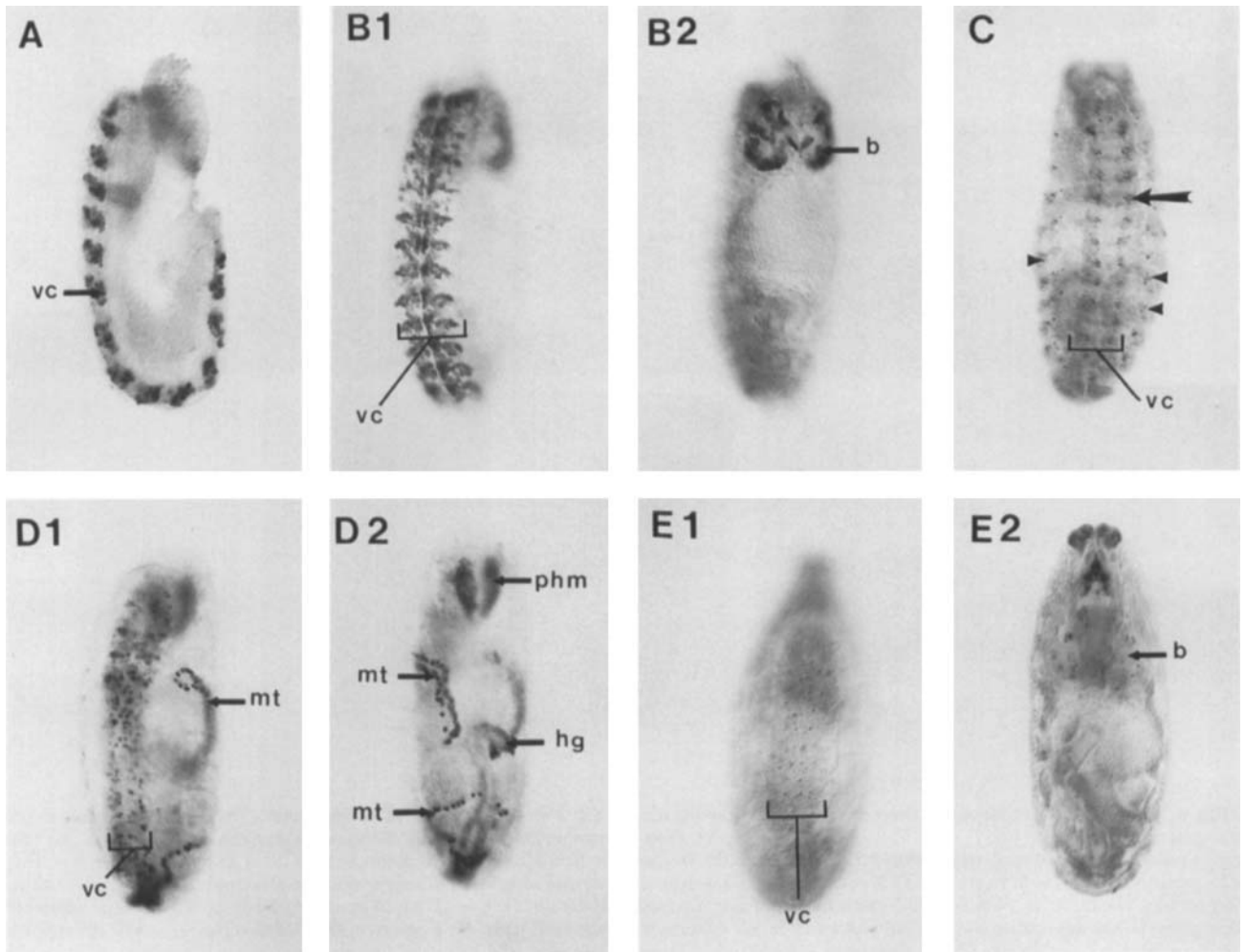
**Fig. 3.** Epidermal expression in various *en-lacZ* strains during embryogenesis. **A:** Insertion line *1-en-14* at 6.5 hr of development, showing 14 intensely stained epidermal stripes. The position of the stripes is in register with those of *engrailed*. **B:** *1-en-18* at 9 hr, this expression pattern is similar to *1-en-14*. **c1:** *1-en-20* at 10 hr; this internal focal plane shows expression in the CNS and some dorsal epidermis. **C2:** *1-en-20*, same embryo as in C1; this more external focal plane shows both the dorsal and ventral epidermal expression. Note that at this stage there is no staining of the lateral epidermis. This pattern is identical to the expression pattern of the segmentation gene *wingless* (van den Heuvel *et al.*, 1989) in which the stripes of staining are anterior to the *engrailed* domain. **D:** *1-en10* at 10 hr of development. Epidermal stripes are present in the *engrailed* domain, but expression is decreased in the ventral and lateral portions of the three thoracic segments. **E1:** *2-en-13* at 9 hr of development. Epidermal expression is confined to the lateral portion of the abdominal segments (arrow).

**E2:** *2-en-13* at 19 hr of development. The lateral staining is only visible on the left side of this embryo since the staining of the other side is out of the focal plane. **F:** *2-en-24* at 17 hr of development. Three stripes of epidermal expression are observed in each segmental unit (bracket) **G:** *1-en-23* at 16 hr of development. This strain labels only the anal pads. **H:** *1-en-28* at 18 hr of development only the posterior spiracles and the most anterior head structures show staining. Nomenclature in this and subsequent figures: Abdominal segments (A), amnioserosa (as), anal pads (ap), anterior midgut (am), brain (b), dorsal epidermis (de), epidermis (e), foregut (fg), hindgut (hg), midgut (mg), malpighian tubules (mt), neuroblasts (nb), neurogenic region (nr), peripheral nervous system (pns), pharyngeal musculature (phm), posterior spiracles (ps), salivary glands (sg), stomodeum (st), thoracic segments (T), trachea (t), ventral cord (vc), ventral midline (vm) and yolk (y). In all figures, anterior is up.

localized within the remaining yolk that is then surrounded by the gut (Fig. 6E). Upon completion of cellularization of the blastoderm, gastrulation begins with the extension of the germ band. It is during germ band elongation that the amnioserosa, constituting the most dorsally located cells, forms. Strain *1A104* specifically labels these cells when they form (Fig. 7A1) until they disappear, following dorsal closure after 12 hr of development (Fig. 7A2).

During gastrulation, cells invaginate midventrally, leading to five different primordia: the anterior midgut, the foregut, the mesoderm, the proctodeum, and the posterior midgut. Cells that invaginate from the ventral furrow give rise to the mesoderm; anterior in-

vagination forms the anterior midgut and foregut, and the posterior invagination forms the proctodeum and posterior midgut. One strain, *1A122*, specifically labels the mesoderm at the blastoderm stage (Fig. 5A), and subsequently labels all mesodermal derivatives. At 9 hr of development, the mesodermal layer is clearly subdivided into somatopleura and splanchnopleura (Fig. 5D). The somatopleural derivatives form the somatic musculature, and the splanchnopleural derivatives give rise to the visceral musculature. During late embryogenesis, all mesodermal derivatives are labeled in strain *1A122*. For example, the dorsal vessel, which develops from two longitudinal rows of mesodermal cells that meet at the dorsal midline after dorsal clo-



**Fig. 4.** A variety of CNS expression patterns obtained with the *en-lacZ* strains. **A:** Insertion line *1-en-65* at 6.5 hr. A subset of cells in the ventral cord and the epidermis are stained. **B1:** *2-en-17* at 9.5 hr exhibits exclusive staining in the CNS and brain. **B2:** *2-en-17*; dorsal view of the brain lobes. **C:** *2-en-11* at 11 hr of development. Two groups of cells in the CNS (arrow) and a subset of cells in the PNS (arrowheads) show expression. **D1:** *2-en-6* at 14 hr of development.

Staining in the CNS is bracketed. The arrow points to expression in malpighian tubules. **D2:** *2-en-6* (same embryo as in D1, different focal plane). Note the expression in the pharyngeal muscles, malpighian tubules, and hindgut. **E1:** *2-en-10* at 16 hr of development. Note the very small number of cells labeling within the nervous system. **E2:** *2-en-10* (same embryo as in E1, different focal plane). Note the small number of stained cells in the brain lobes and the head.

sure, is very darkly stained (Fig. 5E). The strain *1A122* was used to study the mutation *snail* (*sna*) [Nusslein-Volhard *et al.*, 1984]. In *sna* mutant embryos, the ventral furrow fails to form at gastrulation, resulting in the absence of all mesodermal derivatives in the mature embryo. In a *sna* embryo carrying the *1A122* strain, only a few cells stain (Fig. 5F). These cells resemble those seen when *sna* embryos are stained with antibodies against  $\beta$ 3-tubulin, a tubulin isotype whose expression is restricted to the mesoderm [Leiss *et al.*, 1988].

The gut is divided into three regions: the foregut and hindgut are solely of ectodermal origin, and the midgut is mainly endodermal in origin. One strain, *1A121*, labels the anterior and posterior midgut as well as all of

its derivatives (Fig. 6A1–4). Strain *2A20* labels the foregut and the hindgut (Figs. 6C1,C2). The gut completely encloses the yolk at 11.5 hr of development and becomes progressively more narrow and more convoluted at later stages. The gut appears to have three “lobes” at approximately 14 hr and it is during this stage that the malpighian tubules and the gastric caecae, anterior evaginations of the midgut, become apparent. Strain *9A8* labels a subset of cells in the midgut (Fig. 6F). We examined embryos derived from females homozygous for the maternal effect mutation *fs(1) Nasrat*<sup>211</sup>. These embryos complete embryogenesis but have deletions of both anterior and posterior structures [Degelmann *et al.*, 1986]. Posteriorly, abdominal segments A8, 9, and 10; the telson; and the proctodeum are

TABLE 2. Strains That Allow the Visualization of Specific Cell Types\*

Strain	Chromosomal segregation	Viability	Tissue type
1A104	2	V	Amnioserosa (Fig. 7)
1A109	2	V	Nervous system (Fig. 7)
1A115	2	V	Vitelophages (Fig. 6)
1A121	2	V	Midgut (Fig. 6)
1A122	3	E	Mesoderm (Fig. 5)
1A180	2	V	Salivary glands (Fig. 10)
1A333	3	V	Epidermis (Fig. 7)
2A2	3	V	Peripheral nervous system (Fig. 7)
2A18	3	V	Midline neuroepithelium (Fig. 8)
2A20	3	V	Hindgut and foregut (Fig. 6)
9A8	2	V	Subset of the gut (Fig. 6)
1-eve-1	3	V	Trachea (Fig. 10)
1-ftz-43	2	V	Midline neuroepithelium (not shown)

\*We have indicated for each insertion strains its chromosomal segregation, its effect on viability and its dominant pattern of expression during embryonic development. The location of some of these insertion lines were determined by in situ hybridization onto polytene salivary gland chromosomes. The localizations are indicated in parenthesis: *1A109* (26A), *1A122* (93F), *1A180* (53D), *2A18* (62A), *1-ftz-43* (52D). Further information on the  $\beta$ -galactosidase staining patterns can be found in the figures cited. V, viable; E, embryonic lethal.

missing. The abnormal development of the foregut and most terminal part of the midgut can be seen by introducing insertions *1A121* and *2A20* into a *fs(1) Nasrat*<sup>211</sup> mutant background (Figs. 6B, 6D).

As a result of gastrulation, the neuroectoderm lies at the ventrolateral surface of the embryo. The neuroectoderm, or neurogenic region, refers to that area of the embryo destined to form the ventral nervous system and ventral epidermis. Strain *1A109* was found to label all neuroblasts that go on to form the central and peripheral nervous system and later all neurons (Figs. 7C1,2). Whereas the neuroblasts segregate from the neurogenic region, epidermal cells, which do not enter the neural pathway, remain at the surface of the embryo. Strain *1A333* specifically labels epidermal cells as well as the amnioserosa and its derivatives (Fig. 7B1,2). Several strains were found to label the midline neuroepithelium, which lies between lateral neuronal precursors. It gives rise to medial neurons of the CNS, the median neuroblasts, the ventral unpaired neurons, and glial-like support cells. Both strains *2A18* and *1-ftz-43* can be used to mark (Table 2, Fig. 8A1-3) the midline neuroepithelium and its derivatives.

Numerous lines express  $\beta$ -galactosidase in the peripheral nervous system. In Figure 7D, we show the expression pattern of only one of them, line *2A2*. The chordotonal neurons of the lateral clusters of abdominal segments A1-A7 are particularly obvious and easy to follow in this strain.

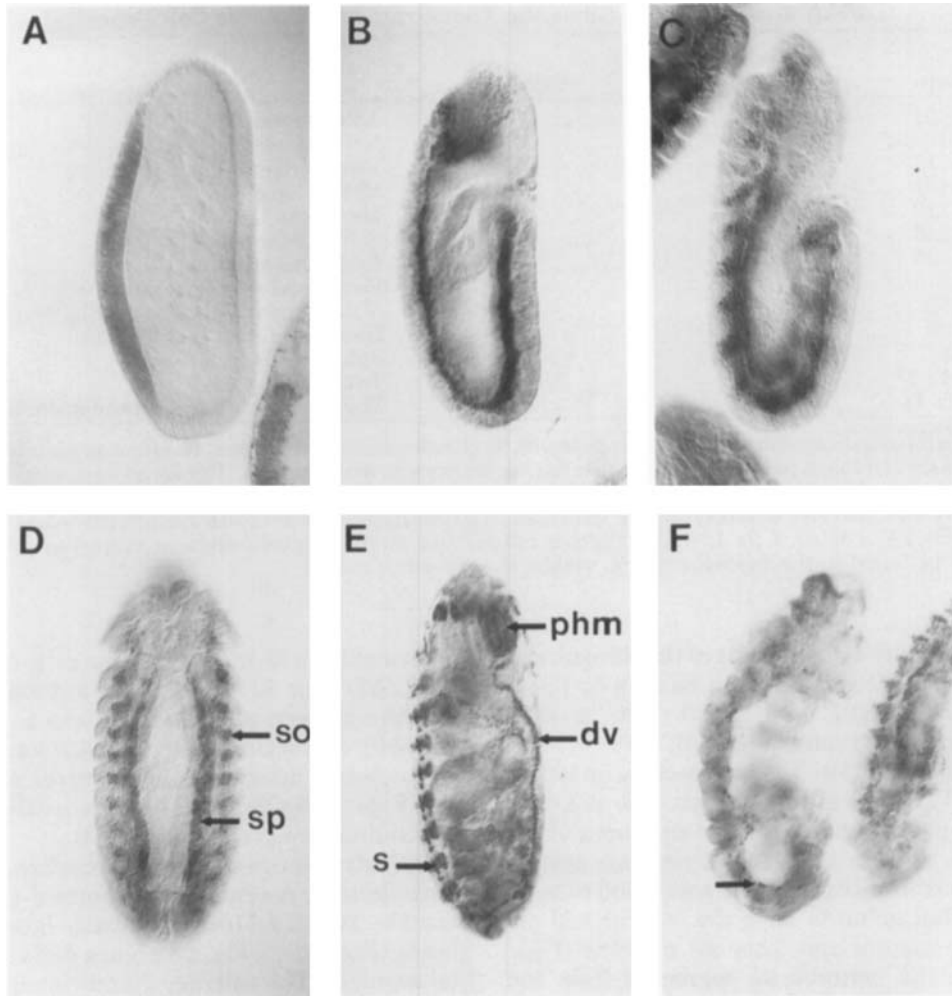
We analyzed the effect of *Notch*, a neurogenic mutation [Wright, 1970; Lehmann *et al.*, 1981], on the pattern of expression of various insertion strains. In a neurogenic embryo, all ventral, lateral, and anterior ectodermal precursors enter the neural pathway at the expense of the epidermis. We examined the expression pattern of transposons that stain neuroblasts (*1A109*),

epidermoblasts (*1A333*), midline cells (*2A18*), and the PNS (*2A2*) (Fig. 9) in *Notch* mutant embryos. In every case, the pattern of expression was as predicted: Hypertrophy of the CNS (Fig. 9A1,A2) and the PNS (Fig. 9B) is clearly detectable, the ventral epidermis is absent (Figs. 9C1,C2), and the cells localized at the ventral midline are present (Fig. 9D).

Many strains can be used to visualize specific subsets of ectodermal, neural, or endodermal derivatives. For example, strain *1A180* specifically labels the salivary glands (Figs. 10C1-3), which are derivatives of the labial segment. The salivary glands invaginate from two lateral placodes on either side of the labial segment that end in the floor of the atrium. We introduced the salivary gland marker, *1A180* (Figs. 10C1-3), into a *giant* mutant background, which has previously been shown to perturb the differentiation of the salivary glands [Petschek *et al.*, 1987]. Loss of zygotic expression of *giant* produces embryos with defects in abdominal segments A5, A6, and A7, and, in addition, the labial lobe and first two thoracic segments are fused [Petschek *et al.*, 1987]. The salivary glands that originate from the labial segments develop abnormally, as can be seen in Figure 10D. In *giant* embryos, the salivary glands appear atrophic, with very few stained nuclei.

One strain, *1-eve-1*, allows the lineage of the tracheal system to be followed (Figs. 10A1-3). An array of 10 segmental placodes from T2 to A8 invaginate laterally to give rise to the tracheal pits, from which the tracheal tree will develop. *even-skipped* embryos, which lack even numbered segments, were used to demonstrate the utility of the tracheal marker *1-eve-1* in following segmental differentiation. As can be seen in Figure 10B, only half the number of tracheal pits are detected.





**Fig. 5.** Expression patterns of *IA122* in the mesoderm and its derivatives. **A:** Ventral expression in the presumptive mesoderm of an embryo at blastoderm stage. **B:** Staining of the mesoderm during germ band elongation at 4 hr. **C:** During germ band retraction at 6 hr of embryonic development mesodermal cells spread laterally and separate into splanchnopleural and somatopleural layers. **D:** At 9 hr the

splanchnopleural musculature surrounds the gut and the somatopleural musculature underlies the epidermis. **E:** Embryo at 20 hr of development. All the differentiated mesodermal derivatives are labeled: somatic muscles, dorsal vessel and pharyngeal musculature are clearly visible. **F:** Expression pattern of strain *IA122* in a *snail* mutant embryo. Note that only a few cells label (indicated by the arrow).

**DISCUSSION**

We have conducted enhancer trap screens to generate lineage-specific patterns of  $\beta$ -galactosidase expression. We screened a total of 1,426 insertion lines and

have identified strains that can be used as markers to label most embryonic cell types.

Among the 1,426 insertion strains that we analyzed, 1,028 were generated using the P[*LArB*] transposon, as used by Bellen *et al.* [1989] and Wilson *et al.* [1989]. We

**Fig. 6.** Development of the gut. **A1:** Insertion line *IA121* at 4 hr of development. Labeling is seen in the primordium of the anterior and posterior midgut. **A2:** *IA121* at 6 hr of development. The two primordia fuse to form the midgut. **A3:** A *IA121* embryo at 11 hr of development (ventral view). Fusion of the midgut primordia has taken place. **A4:** *IA121* at 19 hr of development. The four constrictions of the midgut are clearly visible. Insertion line *IA121* also shows a striped epidermal staining, which is weaker than the expression in the gut (A2, 3, 4). **B:** *IA121* expression in a 12-hr-old mutant embryo derived from females homozygous for the maternal effect lethal mutation *fs(1)N<sup>211</sup>*. The most posterior part of the midgut is defective (arrow),

correlating with the lack of all posterior endodermal derivatives observed in these mutant embryos [Degelmann *et al.*, 1986]. **C1:** Insertion line *2A20*, at 4 hr of development, labels the primordium of the foregut and the hindgut. **C2:** *2A20* at 11 hr of development. Note that the midgut shows no expression of this insertion. **D:** *2A20* expression in 13 hr mutant embryos derived from homozygous *fs(1)N<sup>211</sup>* females. All the derivatives of the hindgut are missing. **E:** Insertion line *IA115* at 16 hr of development. The remaining unbroken yolk nuclei (or vitellogophages) can be seen inside the gut. **F:** Insertion line *9A8* at 22 hrs of development. A very small portion of the midgut labels in the mature embryo.

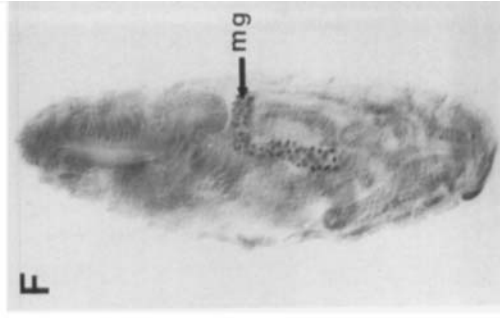
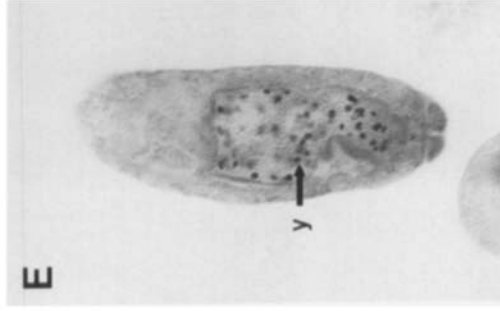
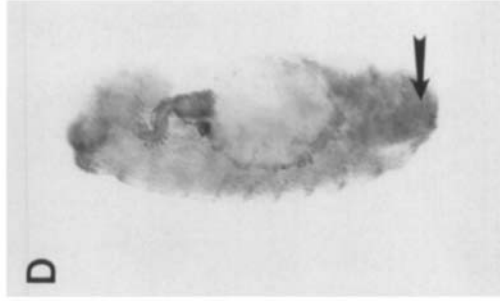
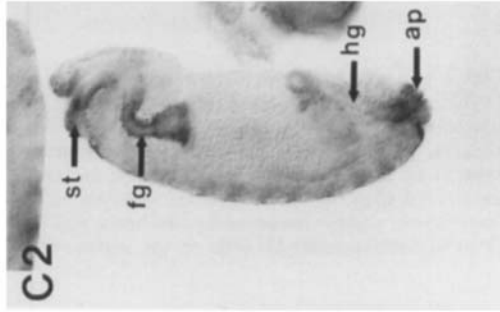
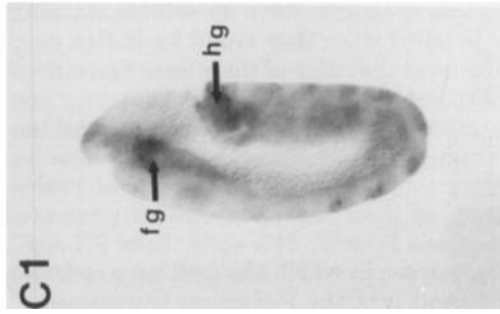
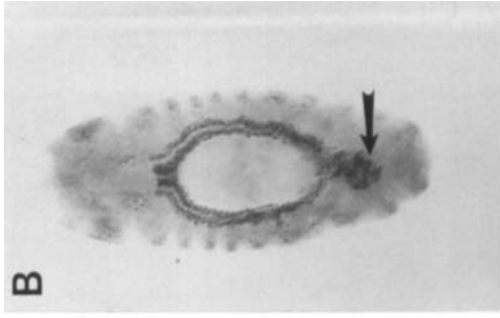
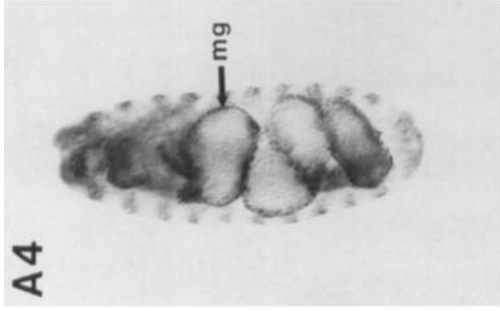
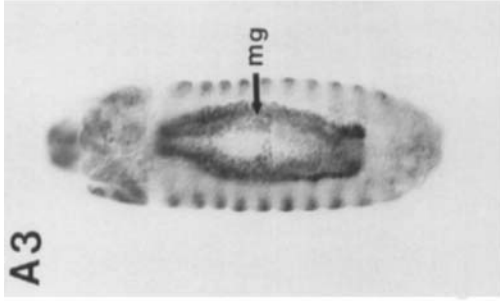
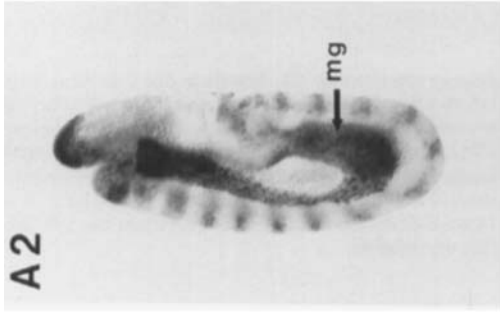
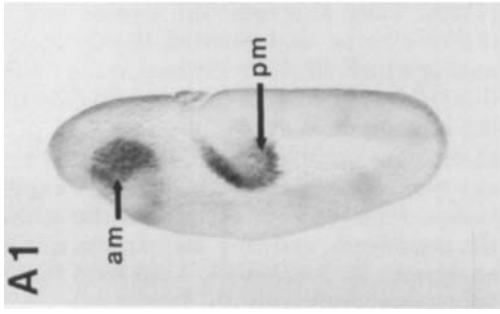
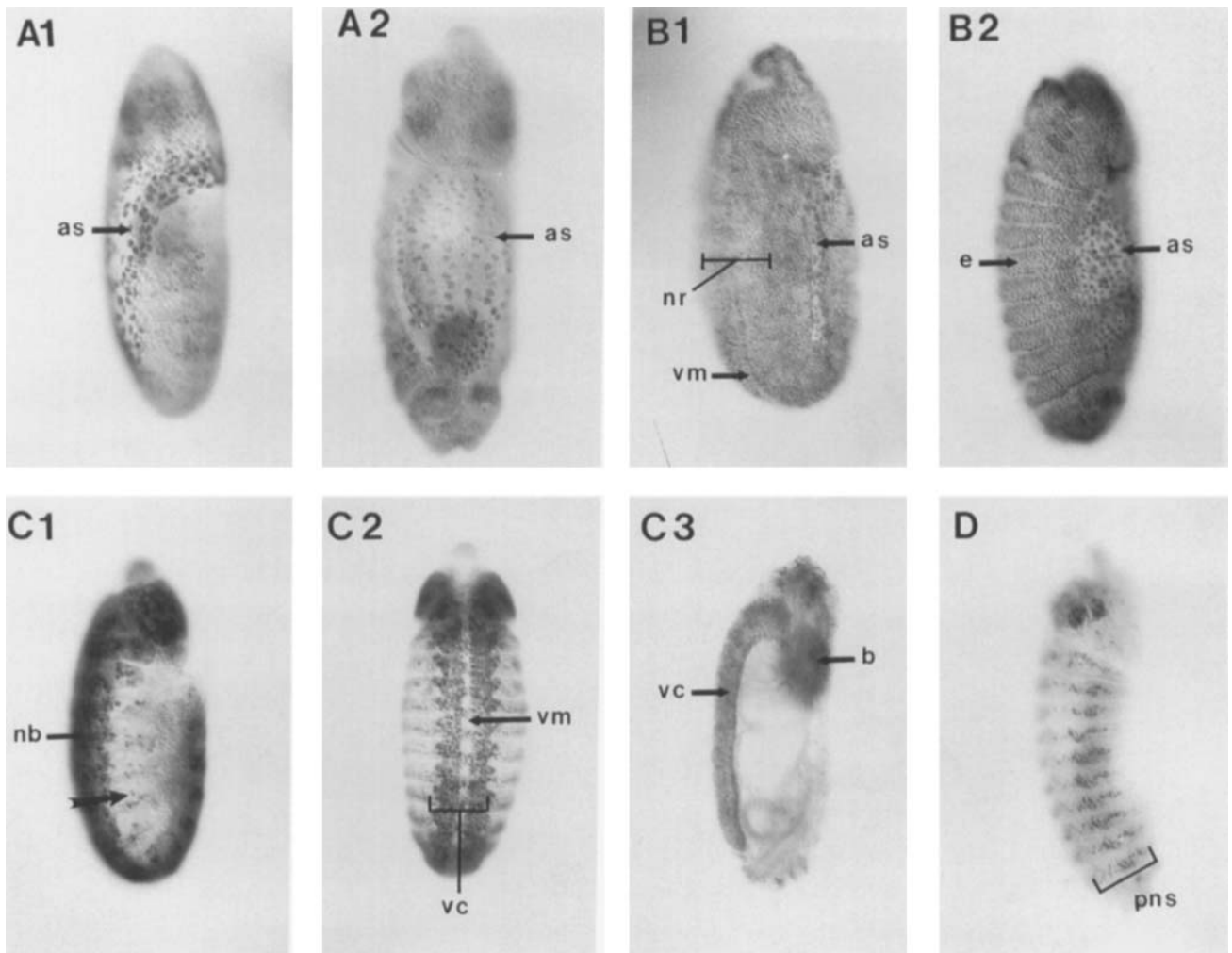


Fig. 6.



**Fig. 7.** Expression patterns in the amnioserosa, epidermis, and neural cells. **A1:** The amnioserosa is stained by insertion line *1A104* at 3.5 hr of development. **A2:** *1A104* at 10 hr of development; dorsal view. **B1:** Insertion line *1A333* label the epidermis, amnioserosa, and all their derivatives at 4.5 hr of development. All cells on the surface of the embryo are labeled, with the exception of the segregating neuroblasts in the neurogenic region (indicated by the horizontal bar). **B2:** *1A333* at 11 hr of development. All cells on the surface of the

embryo are stained. **C1:** Insertion line *1A109* at 5 hr of development labels all segregating neuroblasts. The positions of the neuroblast precursors of the peripheral nervous system is indicated by the arrow. **C2:** *1A109* at 10 hr of development. The ventral nerve cord and brain labels intensely. **C3:** *1A109* at 18 hr of development. The condensing ventral nerve cord and the brain are stained heavily. **D:** Strain *2A2* at 12 hr of development. Clusters of cells in the peripheral nervous system are labeled.

find that 65% of our strains have detectable staining. This number is lower than that found by Bellen *et al.* [1989], who reported that 95% of their insertion strains showed  $\beta$ -galactosidase activity. This discrepancy may be due to the different  $\beta$ -galactosidase assays used (enzymatic vs. immunohistochemical staining). Our results, however, are similar to those of Bier *et al.* [1989], who found that, of their insertion strains generated with the transposon P-lacW, 64% stain. Like P[LArB], P-lacW is a transposon in which the *lacZ* gene is driven by the weak promoter of the P-element transposase.

The distribution of tissue-specific patterns that we observe is comparable to that reported by Bellen *et al.*

[1989]. They analyzed 537 strains and recovered 41 (7.6%) strains that showed highly specific staining, among which 33 (80%) express in the CNS and/or PNS, five (12.5%) express in the gut, and only three (7.5%) in the mesoderm. We find that only 4.8% of our insertion strains are highly specific, i.e., limited to only one tissue type. Among these strains, 61% express in neural tissue, whereas 23% express in the endoderm, 8% in the mesoderm, and only 4% express specifically in the epidermis. In conclusion, it appears that the P[LArB] transposon preferentially detects enhancers that direct neural expression, followed by those that drive endodermal, then mesodermal, and finally epidermal ex-

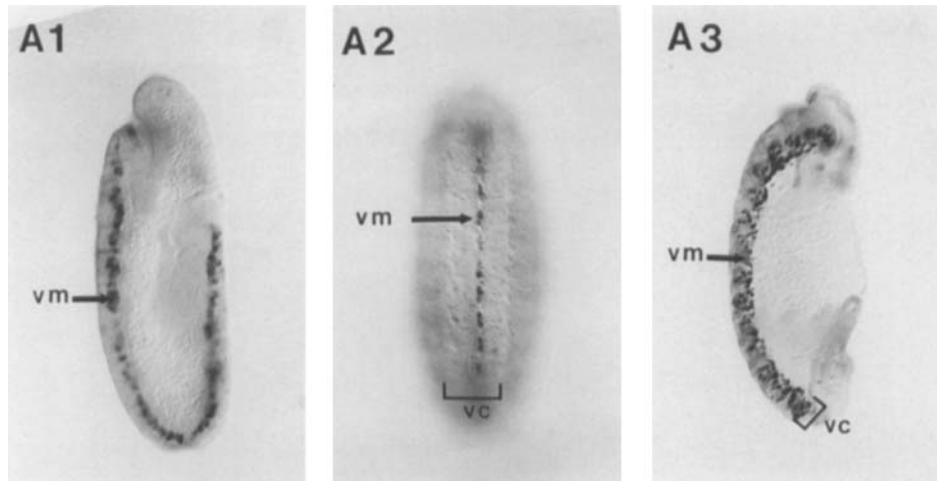


Fig. 8. Strain in which the midline neuroepithelium stain. Strain 2A18 labels cells along the ventral midline. A1: Embryo at 4.5 hr of development. A2: Ventral view of an embryo at 10 hr. A3: Sagittal view of the same embryo.

pression. A similar distribution of expression patterns was observed by Bier *et al.* [1989] using P-lacW.

We characterized in detail 13 lines that can be used as lineage-specific markers for the mesoderm and its derivatives, the gut, the tracheal system, the salivary glands, and the nervous system. These markers allow cell lineages to be followed in both wild-type and mutant embryos. To demonstrate their use in studying mutants, we crossed several of the strains into a variety of mutant backgrounds. The phenotypes of the various mutants can be used to predict the altered expression patterns of the markers. The altered  $\beta$ -galactosidase expression patterns that we observe in mutant backgrounds are close to those predicted from the mutant phenotypes.

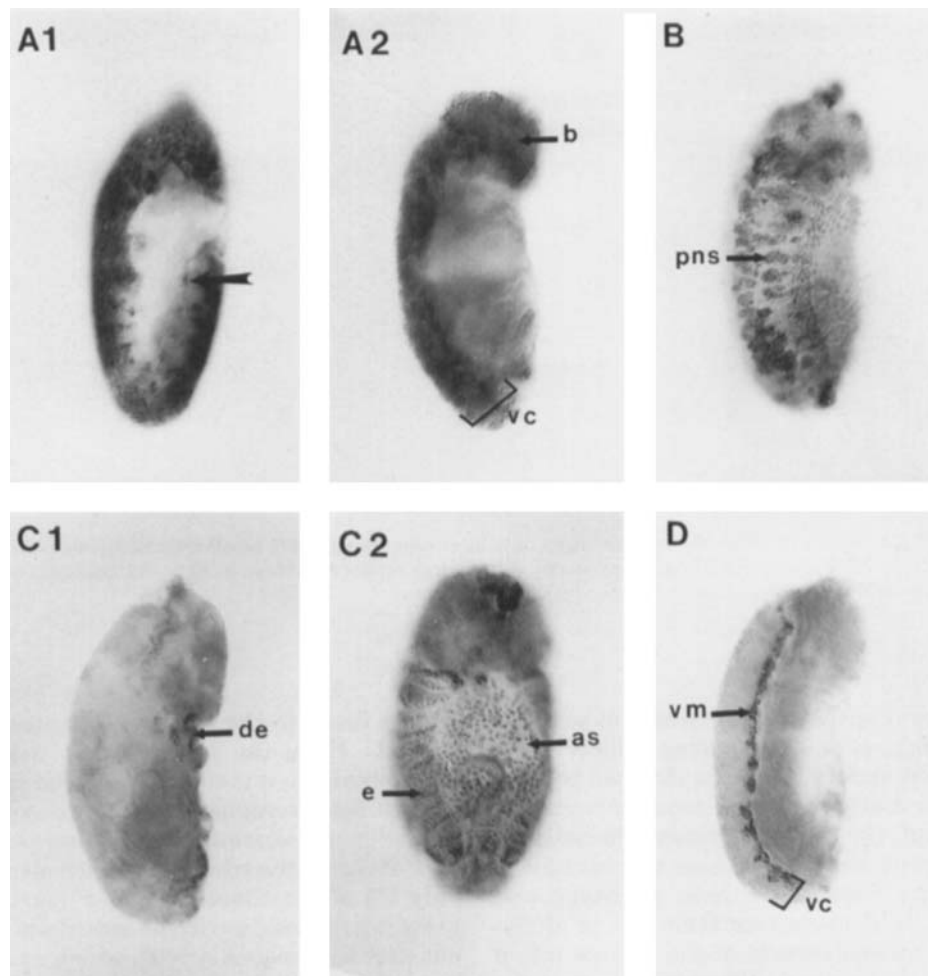
The lineage patterns that we observe may reflect the expression patterns of the genes adjacent to the insertion sites of the P-transposon. Bellen *et al.* [1989] propose that at least six of the 68 insertions that they mapped exhibit the expression patterns of nearby genes. Wilson *et al.* [1989] and Bier *et al.* [1990] have shown that the pattern of  $\beta$ -galactosidase expression of some insertion strains correlates well with the transcription pattern of nearby known genes. We have observed patterns of  $\beta$ -galactosidase expression that resemble the expression of the genes *rhomboid* (using a P[LArB] construct; unpublished data), *wingless* (using the en-lacZ transposon; unpublished data), and *slit* (using the ftz-lacZ construct; unpublished data). In each of these cases, the location of the P-element coincides with the map position of the gene.

To assay whether we could alter this distribution of tissue-specific expression using various transposons, we mobilized three transposons that carry partial pro-

motors fused to the *lacZ* gene (*ftz-lacZ*, *eve-lacZ*, and *en-lacZ*). From the distribution of tissue-specific patterns obtained, it is clear that tissue-specific transcription varies according to the transposon utilized (Table 1). For example, mesodermal patterns are exhibited by 57% of the tissue-specific *eve-lacZ* strains. In contrast, only 8% of the tissue specific P[LArB] insertions express  $\beta$ -galactosidase in the mesoderm. It is also apparent that the frequency with which specific patterns of  $\beta$ -galactosidase expression are recovered depends on the transposon used for generating the insertion strains. Only 4.8% of the P[LArB] strains, 11% of the *ftz-lacZ* strains, and 7.7% of the *eve-lacZ* strains exhibit highly specific patterns of  $\beta$ -galactosidase expression, whereas 41% of the *en-lacZ* strains show tissue-specific patterns. Approximately 10% of all *en-lacZ* strains show  $\beta$ -galactosidase staining in the epidermis and CNS that resembles the pattern of expression of the *engrailed* gene. Possibly, the *engrailed-like* expression patterns of these insertion strains can be explained as augmenting the preexisting patterning element(s) within the *engrailed* promoter. However, since most strains generated with the *en-lacZ* transposon have novel expression patterns of  $\beta$ -galactosidase (Figs. 3C,E,F and 4), this explanation is not sufficient to account for the pattern diversity generated with this transposon.

The high efficiency of detecting enhancers with the three transposons carrying promoters suggests that these constructs detect regulatory sequences that are at least 4 kb away, since the location of the promoter region driving *lacZ* is approximately in the middle of the P-element (Fig. 1).

When partial promoters are fused to the *lacZ* gene,



**Fig. 9.** Analysis of *Notch* (*N*) mutant embryos. **A1:** *IA109* expression in a *N* mutant embryo at 4.5 hr of development (compare with Fig. 7C1). The positions of the neuroblast precursors of the peripheral nervous system is indicated by the arrow. **A2:** *IA109* expression in a *N* embryo at 12 hr of development. Note the enlarged brain and nerve cord. **B:** *2A2* expression in an *N* embryo at 12 hr (compare with Fig.

7D). **C1:** *1A333* expression in a *N* embryo at 12 hr; lateral view. Note that only a small piece of dorsal epidermis remains. **C2:** *1A333* expression in a *N* embryo at 12 hr; dorsal view (compare with Fig. 7B2). **D:** *2A18* expression in a *N* embryo at 11 hr. Interestingly, cells at the ventral midline are not grossly disorganized (compare with Fig. 8A3).

transcriptional specificity may arise from an interaction between genomic enhancers and enhancers within the P-element. Such interactions appear to account for the transcriptional specificities obtained with the *eve-lacZ* and the *ftz-lacZ* constructs. For example, the *eve-lacZ* construct may carry, or preferentially interact with, a mesoderm-specific enhancer. The recovery of insertion strains generated with the *eve-lacZ* transposon can thus facilitate searches for mesodermal markers.

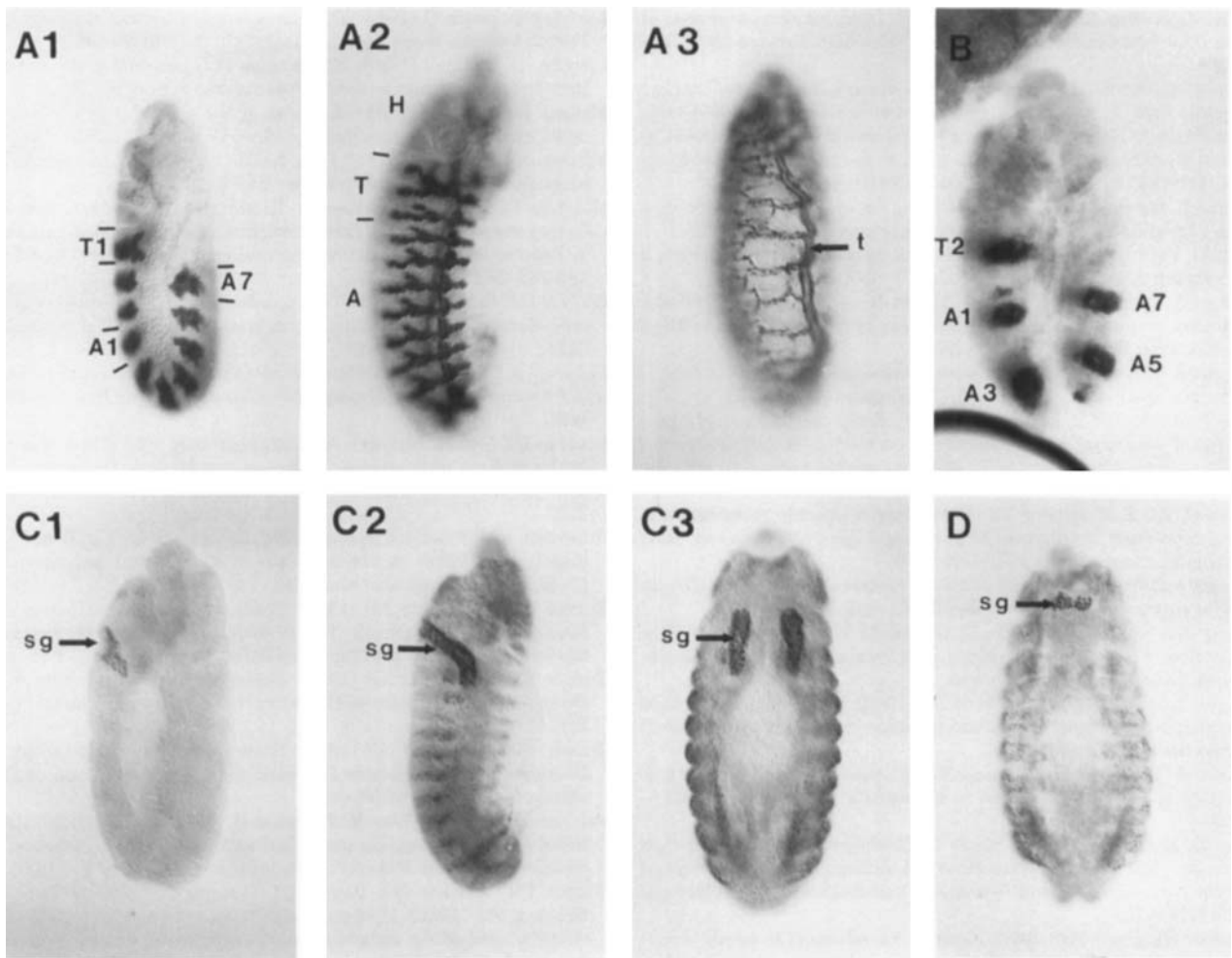
Interactions between genomic enhancers and the enhancers within the P-element may not fully explain the wealth of specific patterns obtained with the *en-lacZ* construct. Mobilization of this element generates a large variety of expression patterns, mainly within the epidermis and the CNS. It is possible that an additional mechanism, such as preferential integration within

particular genes or control sequences, is operating. In situ hybridization to polytene chromosomes to map the sites of insertion of these P-elements supports this hypothesis [Hama *et al.*, 1990; Kassis *et al.*, 1991].

In conclusion, we have demonstrated that insertion strains generated with P-element transposons that carry various sequences upstream of the *lacZ* gene exhibit an increased variety of expression patterns for studying *Drosophila* development. These new patterns may be generated either by combinatorial control between genomic enhancers and enhancers within the P-element or by targeted insertion of P-elements.

#### ACKNOWLEDGMENTS

We thank H. Bellen, U. Grossniklaus, W. Gehring, J. Kassis, C. Dearolf, J. Topol, T. Goto, W. Engels, L.



**Fig. 10.** Patterns of  $\beta$ -galactosidase expression in the tracheal system and salivary glands. **A1:** Strain *1-eve-1* at 6 hr of development labels the invaginating cells from the tracheal placodes. **A2:** *1-eve-1* at 10 hr of development. The tracheal cells migrate along the dorso-ventral and antero posterior axis. **A3:** *1-eve-1* at 22 hr. In the mature embryo, the tracheal tree consists of two main trunks, which run along the anteroposterior axis, connected to many secondary branches, which run along the dorso-ventral axis. **B:** *1-eve-1* expression in an *even-skipped* mutant embryo at 6 hr of development. Only

half the number of tracheal placodes can be detected. Note that this insert exhibits cytoplasmic staining. **C1:** Strain *1A180* at 5 hr of embryonic development. The salivary glands have just begun to form (arrow). **C2:** *1A180* at 9 hr. Note the opening at the level of the labial segment. **C3:** *1A180* at 13 hr; dorsal view. Some tracheal and epidermal staining can also be detected. **D:** *1A180* expression in a *giant* mutant embryo at 13 hr. Note the presence of atrophic salivary glands in *giant* mutant embryos.

Cooley, and the Bowling Green stock center for fly stocks and for stimulating discussions and L. Perkins and E. Siegfried for helpful comments on the manuscript. A.B. was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation. This work was supported by the Howard Hughes Medical Institute.

**REFERENCES**

Bellen HJ, O’Kane C, Wilson C, Grossniklaus U, Pearson RK, Gehring WJ (1989): P-element-mediated enhancer detection: A versatile method to study development in *Drosophila*. *Genes Dev* 3: 1288–1300.

Bienz MG, Soari G, Tremml J, Muller B, Züst, Lawrence PA (1988): Differential regulation of *Ultrabithorax* in two germ layers of *Drosophila*. *Cell* 53:567–576.  
 Bier EH, Jan LV, Jan YN (1990): *Rhomboid*, a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev* 4:190–203.  
 Bier E, Vaessin H, Shepherd S, Lee K, McCall K, Barbel S, Ackerman L, Caretto R, Uemura T, Grell E, Jan LY, Jan YN (1989): Searching for pattern and mutation in the *Drosophila* genome with a P-lac vector. *Genes Dev* 3:1273–1287.  
 Boulet A, Scott M (1988): Control elements of the P2 promoter of the *Antennapedia* gene. *Genes Dev* 2:1600–1614.  
 Campos-Ortega JA, Hartenstein V (1985): “The Embryonic Development of *Drosophila melanogaster*.” New York/Berlin: Springer-Verlag.

- Cooley L, Keeley R, Spradling A (1988): Insertional mutagenesis of the *Drosophila* genome with single P-elements. *Science* 239:1121-1128.
- Dearolf CR, Topol J, Parker CS (1989): Transcriptional control of *Drosophila fushi tarazu* zebra stripe expression. *Genes Dev* 3:384-398.
- Degelmann A, Hardy P, Perrimon N, Mahowald AP (1986): Developmental analysis of the torso-like phenotype in *Drosophila* produced by maternal effect locus. *Dev Biol* 115:479-489.
- Fasano L, Kerridge S (1988): Monitoring positional information during oogenesis in adult *Drosophila*. *Development* 104:245-253.
- Gall JG, Pardue ML (1971): Nucleic acid hybridization in cytological preparation. *Methods Enzymol* 21:470-480.
- Ghysen A, Dambly-Chaudiere C, Aceves E, Jan LY, Jan YN (1986): Sensory neurons and peripheral pathways in *Drosophila* embryos. *Roux Arch Dev Biol* 195:281-289.
- Ghysen A, O'Kane C (1989): Neural enhancer-like elements as specific cell markers in *Drosophila*. *Development* 105:35-52.
- Goto T, Macdonald P, Maniatis T (1989): Early and late periodic patterns of *even skipped* expression are controlled by distinct regulatory elements that respond to different spatial cues. *Cell* 57:413-422.
- Hama C, Ali Z, Kornberg TB (1990): Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. *Genes Dev* 4:1079-1093.
- Kassis JA (1990): Spatial and temporal control elements of the *Drosophila engrailed* gene. *Genes Dev* 3:433-443.
- Kassis JA, Noll E, VanSickle E, Odenwald W, Perrimon N (1991): Altering the insertion specificity of a *Drosophila* transposable element. Submitted for publication.
- Langer-Safer PR, Levine M, Ward DC (1982): Immunological method for mapping genes on *Drosophila* polytene chromosomes. *Proc Natl Acad Sci USA* 79:4381-4385.
- Lehmann R, Dietrich U, Jimenez F, Campos-Ortega JA (1981): Mutations of early neurogenesis in *Drosophila*. *Roux Arch* 190:226-229.
- Leiss D, Hinz U, Gasch A, Mertz R, Renkawitz-Pohl R. (1988):  $\beta 3$  tubulin expression characterizes the differentiating mesodermal germ band layer during *Drosophila* embryogenesis. *Development* 104:525-531.
- Lindsley DL, Grell EH (1968): "Genetic Variations of *Drosophila melanogaster*." Washington, DC: Carnegie Institution of Washington Publ. No. 627.
- Lindsley D, Zimm G (1985): The genome of *Drosophila melanogaster* Part 1: Genes A-K. *Drosophila Inform Serv* 62:181-201.
- Lindsley D, Zimm G (1986): The genome of *Drosophila melanogaster* Part 2: Lethals, maps. *Drosophila Inform Serv* 64:29-36.
- Lindsley D, Zimm G (1987): The genome of *Drosophila melanogaster* Part 3: rearrangements. *Drosophila Inform Serv* 65:6-29.
- Mismer D, Rubin G (1988): Analysis of the promoter of the *ninaE* opsin gene in *Drosophila melanogaster*. *Genetics* 116:565-578.
- Mitchison TJ, Sedat J (1983): Localization of antigenic determinants in whole *Drosophila* embryos. *Dev Biol* 99:261-264.
- Nusslein-Volhard C, Wieschaus E, Kluding H (1984): Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. 1. Zygotic loci on the second chromosome. *Roux Arch Dev Biol* 193:267-282.
- O'Kane CJ, Gehring WJ (1987): Detection in situ of genomic regulatory elements in *Drosophila*. *Proc Natl Acad Sci USA* 84:9123-9127.
- Petschek J, Perrimon N, Mahowald AP (1987): Region specific effects in *U1giant* embryos of *Drosophila melanogaster*. *Dev Biol* 119:175-189.
- Poulson DF (1950): Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meigen. In Demerec M (ed): "Biology of *Drosophila*." New York: Hafner Publ. Co., pp 168-270.
- Robertson HM, Preston CR, Phillis RW, Johnson-Schlitz D, Benz WK, Engels WR (1988): A stable source of P-element transposase in *Drosophila melanogaster*. *Genetics* 118:461-470.
- Turner FR, Mahowald AP (1976): Scanning electron microscopy of *Drosophila* embryogenesis. I. The structure of egg envelopes and the formation of the cellular blastoderm. *Dev Biol* 50:95-108.
- Turner FR, Mahowald AP (1977): Scanning electron microscopy of *Drosophila* embryogenesis. II. Gastrulation and segmentation. *Dev Biol* 57:403-416.
- Turner FR, Mahowald AP (1979): Scanning electron microscopy of *Drosophila* embryogenesis. III. Formation of the head and caudal segments. *Dev Biol* 68:96-109.
- van den Heuvel M, Nusse R, Johnston P, Lawrence PA (1989): Distribution of the *wingless* gene product in *Drosophila* embryos: A protein involved in cell-cell communication. *Cell* 59:739-749.
- Wilson CR, Tearson HJ, Bellen CJ, O'Kane CJ, Grossniklaus U, Gehring WJ (1989): P-element mediated enhancer detection: An efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. *Genes Dev* 3:1310-1313.
- Wright TRF (1970): The genetics of embryogenesis in *Drosophila*. *Adv Genet* 15:262-395.