Development 117, 1287-1297 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Gene expression during imaginal disc regeneration detected using enhancer-sensitive P-elements

William J. Brook, Lisa M. Ostafichuk, Jana Piorecky, Mark D. Wilkinson, Daralyn J. Hodgetts and Michael A. Russell

Department of Genetics, University of Alberta, Edmonton, Alberta, CANADA T6G 2E9

SUMMARY

When imaginal disc fragments from *Drosophila* are cultured in adult female hosts, they either duplicate the part of the pattern specified by the fate map, or regenerate to replace the missing part. The new tissue is added by proliferation of a small number of cells from the cut edge, brought together when the wound heals to form a regeneration blastema. Specification of the new pattern has been explained by assuming interactions among cells of different positional value in the regeneration blastema. In order to identify genes which might mediate these events, we screened over eight hundred independently isolated autosomal insertions of an enhancer-sensitive P-element, for altered *lac-z* expression in regenerating discs following cell death induced by a temperature-sensitive cell-lethal mutation. Two further screens divided the positive lines into four groups based on appropriate timing of the *lac-z* response in the cell-lethal mutant background and the expected response to an alternate source of cell death. Expression in wing disc fragments cultured in vivo was most frequent in the target class defined by the screens. In this direct test, *lac-z* expression was found in 23 lines and in most cases was spatially and temporally correlated with the formation of the regeneration blastema. Our results suggest a very substantial transcriptional response during the early stages of imaginal disc regeneration. *lac-z* expression in control imaginal discs, embryos and adult ovaries of the positive lines was also assayed. The selected insertions included: a small class expressed only in discs undergoing regeneration and apparently not at any other stage, possibly representing genes active exclusively in regeneration; a larger class expressed in the embryo or during oogenesis, but not normally in imaginal discs, as expected for functions recruited from earlier stages of the developmental program; and finally a class with spatially patterned expression in normal discs. This class included several insertions with expression associated with compartment boundaries, including one at the *decapentaplegic (dpp)*, and one at the *crumbs (crb)* locus, a growth factor homologue, and an EGF-repeat gene respectively. Some of the expression patterns observed in cultured disc fragments provide evidence for cell communication in the regeneration blastema.

Key words: imaginal discs; regeneration; enhancer detection, *Drosophila*

INTRODUCTION

The adult cuticular structures of *Drosophila* differentiate at metamorphosis from segmentally derived single-layered epithelial invaginations called imaginal discs. Each disc normally forms a fixed portion of the pattern of cuticular elements of the corresponding segment, but the developmental fates of individual cells are not irreversibly determined, and the evidence suggests that the fate of a cell can be influenced both by its lineage history or compartmental cell-state (Garcia-Bellido et al., 1973) and by positional information (Wolpert, 1969).

When complementary imaginal disc fragments are cultured in vivo, one fragment can regenerate the part of the pattern that was deleted, while the other can only duplicate the part it normally forms in situ (Schubiger, 1971). Thus the new partial pattern added to each fragment is identical, but its extent differs depending on the location of the cut. This suggested that the new pattern might be specified by positional information at the cut edge common to both fragments (Bryant, 1971; Postlethwaite and Schneiderman, 1973). The way the new pattern is generated in each disc fragment involves folding of the cut edge and healing to restore continuity of the epithelial surface across the wound. The patterns regenerated in a wide variety of experiments have been explained by postulating strictly local interactions between the cells brought together in this way (French et al., 1976; Bryant et al., 1981). According to this model, positional disparities across the wound stimulate local cell proliferation, resulting in the interpolation of the missing intermediate positional values.

Clonal labelling of disc cells at the time these events are initiated (Girton and Russell, 1980; Abbott et al., 1981), showed that the new pattern is derived from a very small

group of cells, presumed to be from the site of the wound and referred to as a regeneration blastema. Further evidence for a distinct cell population at this site is the localized pattern of BUdR incorporation and cell division seen there (Bryant and Fraser, 1988). Clonal analysis of duplicating leg discs showed that the normally cell-heritable anterior-posterior (A/P) compartmental commitments are reprogrammed in the regeneration blastema (Girton and Russell, 1981; see also Abbott et al., 1981; Szabad et al., 1979, for similar results with regenerating fragments). Remarkably, this event in the regeneration blastema reiterates one that occurs normally in response to segmental positional cues in the early embryo (Ingham, 1988; Vincent and O'Farrell, 1992), suggesting possible common steps in the pathways for embryonic establishment and regeneration of pattern in imaginal discs.

What is the relationship between positional information and compartmental cell-state? Segmental positional information may be encoded in the repeating stripe expression pattern of the segment polarity genes in the germ band stage embryo (Russell, 1985; Martinez-Arias, 1989). Several segment polarity genes are also expressed in compartmentally defined patterns in the imaginal discs, but often not in patterns obviously related to the embryonic stripe pattern (Kornberg et al., 1985; Baker, 1988; Phillips et al., 1990; Eaton and Kornberg, 1990; for review, see Wilkins and Gubb, 1991). Meinhardt (1983) has suggested a modification to the model of French et al. (1976) in which compartment boundaries in imaginal discs act as discrete local organizers of continuous global gradients of positional information.

To explore these issues we have attempted to identify the genes involved, by looking for altered expression in regenerating discs. The screen was designed to detect such loci by differential expression of an enhancer-sensitive reporter gene carried on a transposable, P-element-derived 'enhancer-trap' construct (O'Kane and Gehring, 1987). Using this strategy we hoped to recover material that would allow us to test the ideas outlined above, without restricting the scope of our investigation to existing models and genes already identified.

MATERIALS AND METHODS

Fly stocks

Most mutations and balancers used in this study are described in Lindsley and Zimm (1992). A stock with an X-chromosome insertion of the PZ enhancer-sensitive element (Jacobs et al., 1989), a stock containing a P[Δ2-3,ry^+](99B) element (Robertson et al., 1988) on a *ry Sb e* third chromosome, a *T(2;3)CyO-TM6* stock, as well as a small number of autosomal insertions of a different enhancer-sensitive P-element (O'Kane and Gehring, 1987), were obtained from J. Merriam. A crb^{MA22} stock and a $Df(3R)crb^{S87\text{-}4}$ stock were obtained from U. Tepass. The *y v f* $su(f)^{12}$ stock (formerly called *l(1)ts726*) originated in this laboratory (Russell, 1974). Flies were grown on a yeast-sucrose-agar medium (Nash and Bell, 1968) at 25°C except for *y v f* $su(f)^{12}$ which was maintained at 22°C.

Enhancer-trap insertions

PZ is an enhancer-sensitive P-element incorporating a P-transposase:*lac-z* fusion, bacterial plasmid sequences and a $rosy^+$ eye-colour marker. Transposant male offspring (phenotypically wild-type for all markers) were selected from crosses between *pr cn/T(2;3)CyO-TM6/mwh ry e* females and single *PZ[lac-z,*ry^+*]/Y; ry Sb e* P[Δ2-3,ry^+](99B)*/ry* males (as in Cooley et al., 1988). To ensure that the selected insertions all arose from independent events, only one transposant was saved from any cross. Transposant males were crossed to *pr cn/T(2;3)CyO-TM6/mwh ry e* females to balance the transposition and the insertions were assigned to the second, third, or fourth chromosomes by analysis of the segregation of ry^+ from the *purple, cinnabar (pr cn)* and/or *ebony (e)* marked chromosomes.

Generation of cell death in the imaginal discs

Cell death was induced using the mutation $su(f)^{12}$, a temperature-sensitive cell-lethal allele of *suppressor of forked* (1-67; Russell, 1974). Males from each insertion line were crossed, at 22°C, to females homozygous for $su(f)^{12}$ (see Fig. 1). The cultures were shifted to 29°C upon appearance of third instar larvae and left for 24 hours, or 48 hours followed by a 24 hour recovery at 22°C, depending on the test to be performed. Larvae were then scored for sex and mouthhook phenotype to separate *y v f* $su(f)^{12}$*/Y ; PZ/+* (yellow, cell-lethal males) and *y v f* $su(f)^{12}$*/+; PZ/+* (wild-type control females). Alternatively, cell death was induced by exposing third instar larvae to 2500 rads of γ-radiation from a ^{60}Co source followed by an 8 hour recovery period (James and Bryant, 1981). Untreated larvae at the same developmental stage were used as controls. In each case, the anterior one-third of 5-10 treated and control larvae was cut off, everted, and the remaining complex stained after discarding the fat body and salivary glands. Tests were scored as positive when the imaginal disc X-gal staining was judged to differ in treated and control larvae.

In vivo culture of wing imaginal disc fragments

Wing discs were dissected from late third instar larvae in insect Ringers and fragmented with a sharpened insect pin in one of three ways (see Fig. 4A). a-a′ is a V-shaped cut through the prospective alar lobe and costa with its apex in the anlage of the ventral wing blade. This yields a bi-lobed ventral fragment similar to B of Dale and Bownes (1985), but the a-a′ cut is more ventral making the angle of the 'v' wider. The b-b′ cut is similar to cut '6' of Bryant (1975), giving a large dorsal fragment. The c-c′ cut produces a small fragment consisting entirely of posterior compartment tissue. These fragments were injected into mated 24-48 hour post-eclosion Canton-S females. Fragments or host abdomens were recovered and assayed for β-galactosidase activity as described below.

β-galactosidase detection

Staining of imaginal discs, embryos and adult ovaries with X-gal was performed essentially as previously described (Simcox et al., 1989; Bellen et al., 1989; Grossniklaus et al., 1989). Immunohistochemical detection of β-galactosidase in cultured disc fragments was performed with a polyclonal rabbit antibody directed against *E. coli* β-galactosidase (Cappel) and the Vectastain Elite kit (biotinylated goat anti-rabbit secondary antibody, avidin-HRP tertiary complex; Vector Labs) in host abdomens, which were detached from the thorax and dissected along the dorsal midline to facilitate access of reagents. The staining procedure was that described by Pattattucci and Kaufman (1991) with only minor adjustments.

Acridine-orange staining

Everted larval heads were transferred to an acridine orange solution (5 μg/ml in Ringers), incubated for 5 minutes, briefly washed in Ringers and photographed immediately under epifluorescence.

RESULTS

Screening for enhancer-traps expressed in imaginal discs after cell death

Because it can detect patterns of gene regulation, an enhancer trap method was used to search for genes whose expression might change in regeneration. The constructs employed carried an *E. coli lac-z* reporter gene, fused in frame to the P-transposase promoter which may be *cis*-activated when the transposon inserts near an enhancer of an active gene. The enhancer trap was mobilized and more than 800 independent autosomal insertion lines were recovered using the mating scheme described in the Materials and Methods. To initiate regeneration in each of these lines without recourse to time-consuming surgical fragmentation and in vivo culture, we made use of *su(f)*^{12}, a temperature-sensitive cell-autonomous lethal allele of *suppressor of forked*, in which genetically induced cell death efficiently produces disc fragments that regenerate and duplicate in situ (Russell, 1974; Clark and Russell, 1977; Girton and Kumor, 1985). Fig. 1 outlines the primary screen. Insertion lines were saved when the staining pattern of discs from cell-lethal males and control females differed, e.g. as in Fig. 1C and D.

A total of 826 autosomal insertion lines was screened for altered expression in the cell-lethal mutant background. The *lac-z* expression of 38% (312/826) of the lines tested was altered in some way. These included insertions expressed only in cell-lethal and not in control discs (82 lines), insertions expressed in an altered pattern (229 lines), and one expressed in controls but not mutant discs. Some examples are shown in Fig. 2. The large class of lines in which a control expression pattern was altered cannot be explained merely by physical distortion of the disc epithelium due to cell death, because equally complex control expression-patterns were unchanged after treatment in most of the lines (e.g. Fig. 2I,J).

In view of the high frequency of positives obtained, further classification of the lines was necessary. Mutants of *suppressor of forked* modify expression of gypsy-element insertion alleles at several loci. It has been reported that *su(f)*$^{+}$ is a negative regulator of gypsy transcription (Mazo et al., 1989). As it is an essential gene, *su(f)* may similarly influence expression of certain normal genes. To see whether any of the responding insertions might be at loci regulated fortuitously by *su(f)*, rather than in genes participating in regeneration, we retested the positives for expression during the heat treatment before regeneration is initiated. Expression was altered in 112 of the lines after 24 hours at 29°C. Since cell death in this system only becomes evident 36 hours after a shift to the restrictive temperature (Clark, 1976), insertions affected as early as 24 hours must be responding directly to the *su(f)*^{12} lesion and not acting as genes participating in regeneration. This left 200 lines in which *lac-z* expression is affected only after regeneration has been initiated.

To test whether the insertions could respond to an alternative source of cell death, as expected if they are indeed in regeneration genes, discs from each of the positive lines were assayed 8 hours after exposure of third instar larvae to a 2500 rad dose of γ-radiation, and compared to discs from untreated larvae at the same developmental stage. It has been estimated that this dose kills 30% of cells in imaginal discs within 4 hours after treatment. By 8 hours, sur-

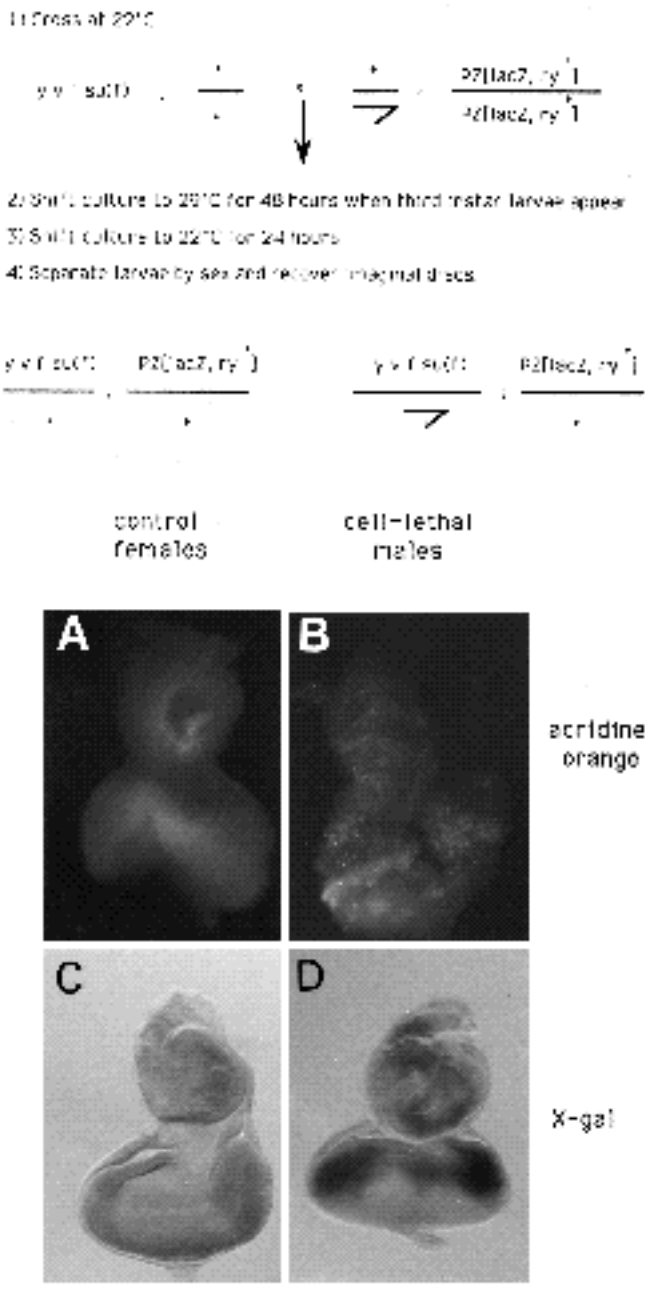

Fig. 1. Crossing scheme used to generate cell-lethal and control larvae carrying PZ insertions. The cross and subsequent treatments were performed for each insertion line. (A) Eye-antenna disc from control female. Localized acridine orange staining in antennal disc indicates a low level of normal programmed cell death (Spreij, 1971). (B) Eye-antenna disc from heat-pulsed *su(f)*^{12} male with greatly increased punctate acridine orange staining indicating cell death induced by the cell-lethal mutation. (C) Eye-antenna disc from heat-pulsed *su(f)*^{12}*/+; D-42/+* females with no detectable β-galactosidase activity. (D) Eye-antenna disc from heat-pulsed *su(f)*^{12}*; D-42/+* male with substantial β-galactosidase activity distributed in a pattern similar to the induced cell death (compare B and D).

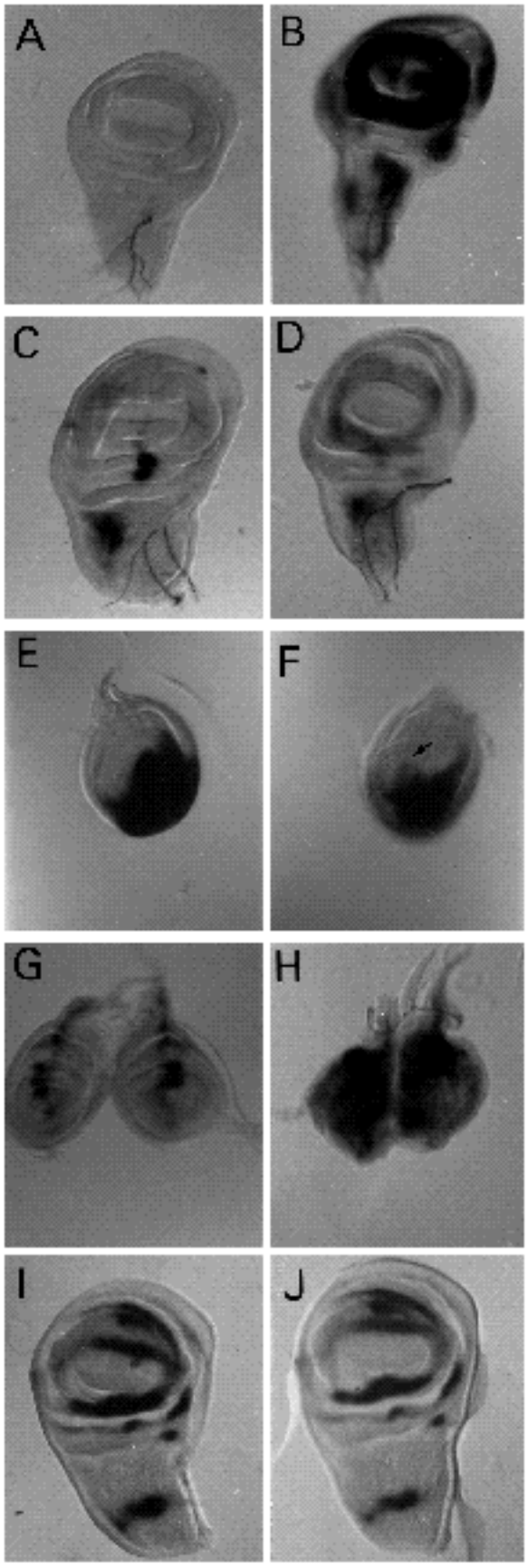

Fig. 2. *lac-z* expression patterns in control (left-hand column) and cell-lethal (right-hand column) discs from five PZ insertion lines. (A) Control and (B) cell-lethal D-42 wing discs showing strong induction of β-galactosidase in a complex pattern throughout the disc. (C) Control B-93 wing disc with strong localized notum expression and in addition weak, punctate expression forming a cross in the wing pouch. (D) Cell-lethal B-93 wing disc with weak β-galactosidase activity in the notum and encircling wing forming region. The cross is no longer visible. (E) Control H-15 metathoracic leg disc with expression apparently restricted to the ventral compartment. (F) Cell-lethal H-15 metathoracic leg with ectopic stain in dorsal tarsus-forming region indicated by the arrow. (G) Control E-32 prothoracic leg discs staining at or near the anterior-posterior compartment boundary. (H) Cell-lethal E-32 prothoracic leg discs with staining greatly expanded along the A/P boundary and additional weak staining remote from the boundary in both anterior and posterior compartments. (I, J) Control and cell-lethal H-09 wing discs with no detectable differences in staining. All single discs are oriented with the anterior comparment to the left. Wing discs are ventral side up; leg discs are ventral side down.

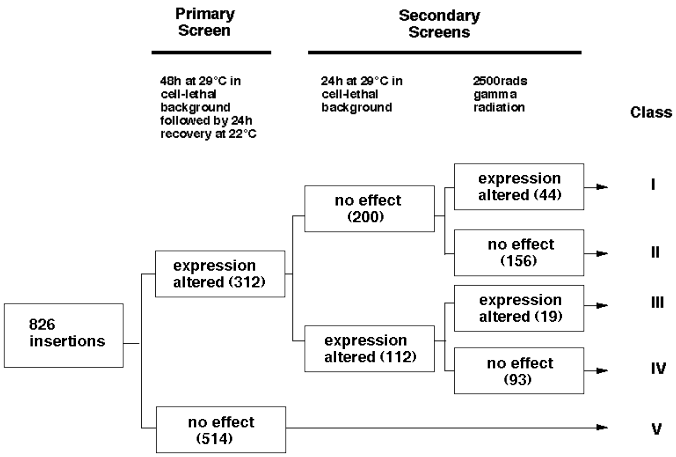

Fig. 3. Definition of the five classes of insertion lines according to the primary and secondary screens. The numbers in parentheses indicate the distribution of the 826 lines according to each test criterion.

viving cells re-enter the cell-cycle and begin to replace lost tissue (James and Bryant, 1981). The *lac-z* expression of 63 lines was judged to be altered by this treatment.

The two secondary screens defined four classes of insertion among those initially selected as positive (Fig. 3). The target class I insertions responded at the appropriate time to both kinds of cell death, but as the times chosen for monitoring *lac-z* expression were necessarily arbitrary, we retained all 312 primary positives pending evaluation of the specificity of the screens, by the tests described below.

Expression patterns in control imaginal discs

Most of the positives also expressed *lac-z* in control discs. Table 1 summarizes our data on the staining patterns of wing, haltere, leg and eye-antenna discs from control larvae. The incidence of different control expression patterns differs strikingly among the five classes of insertion defined by the screens.

The most frequent control expression pattern among class V (non-responding) lines is uniform, accounting for 47% of the sample scored. This pattern is much less frequent among the positive lines, especially the target class I, where it accounts for only 5% of the total. The next most common pattern in control discs is non-expression (Fig 2A). This category is again under-represented in the target class (9% of class I but 27% of all other lines). The remaining lines are expressed in spatially non-uniform patterns in control discs (Fig. 2C,E,G and I). Interestingly, this category is much more common among lines that respond to the primary screen, and the over-representation is most marked in the target class (86% of class I, 67% of class II, 53% of III, 59% of class IV, and 25% of class V). These results suggest that pre-existing patterned gene expression in the imaginal discs may be important for pattern respecification in regeneration.

The particular expression patterns found preferentially in the target class were as follows (see Table 1). The most common was a pattern that included corresponding parts of distal antenna and leg. Interestingly, most of these lines also expressed *lac-z* at or posterior to the morphogenetic furrow in the eye-disc (e.g. Fig. 5C). The incidence of this expression pattern was enhanced about five-fold among insertions that responded to the primary screen. In another group of lines, control expression was in discrete spots along the putative wing margin and/or notum in patterns similar to proneural and neurogenic genes involved in development of the imaginal sensory nervous system (e.g. Fig. 2C). This pattern was about twice as common among the selected lines. Finally, two groups of lines were expressed in control discs in patterns correlated with the compartmental subdivisions. Compartmental expression patterns (e.g. Fig. 2E) were about equally frequent in responding and non-responding classes, but 15 of the 16 lines that showed expression along a compartment boundary (e.g. Figs 2G, 4A) were found in the target class I or class II. For several of the lines (AD-55, E-32 and H-39) the approximate A/P boundary site of expression was confirmed by their coincident expression in double-insertion combinations with each other and with the *engrailed* enhancer-trap insertion, ryXho25 (Hama et al., 1990).

Expression during in vivo culture of disc fragments

In vivo culture of a surgical disc fragment in an adult female host is the most direct test for expression of an insertion during pattern regulation. Use of a defined cut instead of random cell death makes it possible to ascertain how expression correlates with the site of the wound, and its time course can be established from staged implants. The *lac-z* expression in wing disc fragments cut as indicated in Fig. 4A, was examined following culture for either 1 or 2 days in adult female hosts. This time interval allows the edges of the fragments to heal together, but precedes the initiation of intercalary cell division (Bryant and Fraser, 1988). Canton-S disc fragments, implanted, recovered and stained as controls, showed no detectable activity in the disc epithelium at the cut edge. In a few cases, these controls showed irregular X-gal staining in material adhering to the surface of the disc (similar to that indicated in Fig. 4F, *). This staining may be in haemocytes or other material from the host.

The examples in Fig. 4 illustrate the features that led us to infer ectopic *lac-z* expression in particular lines. Fig. 4A is an intact H-39 wing disc showing control expression along the A/P compartment boundary. Fig. 4B shows a cultured ventral a-a′ fragment oriented to correspond with the intact disc in Fig. 4A (note weak control expression at *). Strong *lac-z* expression (bracketed region) is only visible at the wound where the cut edges have begun to heal. This interpretation is consistent with the new pattern of folds and change in shape of the original bilobed fragment. The bracketed region is enlarged in Fig. 4C where *lac-z* expression is clearly visible in two distinct epithelial cell layers juxtaposed across a morphological discontinuity (arrows). Since no such discontinuity can be seen along the compartment boundary where *lac-z* is expressed in intact discs (Fig. 4A), we interpret this to be ectopic expression in a and a′ cells brought together at the wound heal. The superficial staining seen sometimes in the Canton-S controls was also occasionally observed in experimental lines (* in Fig. 4F) but was quite distinct from staining in the disc epithelium. Localized expression in cell nuclei next to prominant epithelial discontinuities (arrows) is also shown in Fig. 4G-

Table 1. Control staining patterns in imaginal discs from insertion lines of each class defined by the screens

lac-z expression pattern*	Class of insertion I (*n*=44)	II (*n*=156)	III (*n*=19)	IV (*n*=93)	V (*n*=375†)	total (*n*=687)
no expression	4	45	7	26	106	188
uniform expression	2	6	2	12	177	199
posterior to morphogenetic furrow in eye disc‡ and distal portions of leg and antenna discs§	17	53	5	29	27	131
distal portions of leg and antenna discs§	8	29	2	20	10	69
proneural (wing margin or discrete spots in discs)	2	5	0	0	3	10
posterior to morphogenetic furrow in eye disc‡	1	3	0	0	6	10
compartment boundary	6	9	0	0	1	16
compartmental	1	3	0	0	5	9
other	3	3	3	6	40	55

*Strains are categorized by the most prominent staining pattern exhibited.
†Data from a sample of class V lines (375 of 514) are shown.
‡Expression in all or most of eye disc posterior to morphogenetic furrow and in a few cases slightly anterior to the furrow as well.
§Similar restricted expression in corresponding folds of leg and antennal discs.

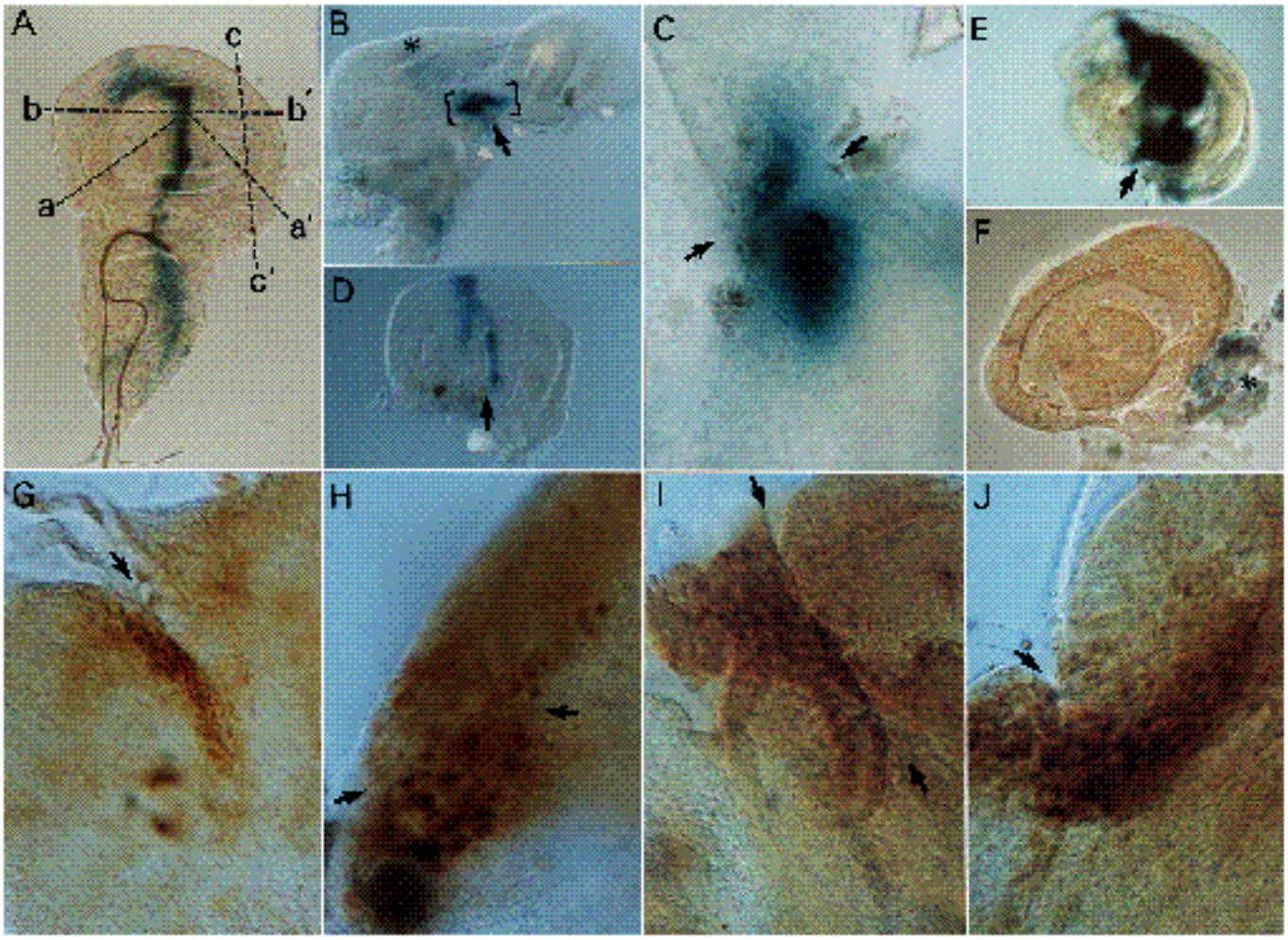

Fig. 4. *lac-z* reporter gene expression in disc fragments after in vivo culture. (A,B and D-F) All discs and fragments are oriented ventral up, posterior to the right. All fragments were cultured for 24 hours. (A) An unoperated H-39 disc with location of cuts indicated by dashed lines (a-a′, b-b′, c-c′). H-39 is expressed strongly at the A/P boundary and very weakly throughout the posterior compartment. (B) Ventral a-a′ H-39 fragment with the arrow indicating strong expression at the putative site of wound healing. Note also the weaker original expression (*). (C) High magnification of bracketed region in B. The strong X-gal stain is confined to within a few cell diameters of an epithelial discontinuity (arrows) representing the wound heal. (D) Ventral a-a′ E-32 fragment. X-gal staining is restricted to one side of the putative wound heal (arrow). Compare with H-39 in C where cells on both sides of the wound heal are clearly stained. (E) Ventral a-a′ AD-55 fragment with strong ectopic staining on both sides of the healed wound (arrow). (F) Posterior c-c′ AD-55 fragment with no detectable staining in the epithelium. Staining material (*) is of uncertain origin but is clearly not epidermal (see text). (G-J) Immunohistochemical detection of β-galactosidase in b-b′ dorsal fragments from three insertions that show no control expression in unoperated discs. In all cases expression in a small number of epithelial cell nuclei is correlated with a discontinuity (arrows) indicating the wound heal. (G) F-36 and (H) E-37 fragments show assymmetric and symmetric staining respectively at the wound heal. (I,J) G-45 staining appears to be assymetrically localized on one side of the wound heal.

J. These are dorsal b-b′ fragments from lines that have no expression in control discs, so the *lac-z* staining visible at the putative wound heal must indeed be ectopic. Another example using a more dorsal cut is interpreted in Fig. 5A,B. Taken together these examples show ectopic expression in cells from diverse original sites, which were brought together by wound healing.

To avoid ambiguities that might arise as a result of control expression in the disc fragment, we applied the in vivo culture test only to lines with no control expression, and to the compartment boundary class, where the expression provides a useful positional marker. From 5 to more than 20 successfully recovered implants were examined for each line, and in positive lines up to about half of these fragments showed ectopic expression. Expression would not be expected in every fragment after 1-2 days in culture, because healing is initiated heterogeneously over a period of several days (Bryant and Fraser, 1988), and not all fragments would ultimately regenerate. The results are summarized in Tables 2 and 3. Ectopic β-galactosidase activity was induced in cultured disc fragments from 23 of the 81 lines tested. The positives included more than half (4/7) of the class I lines tested, and all but one of the remainder (18/54) were from class II, further vindicating the design of the screens. A single exceptional line (C-39) from class III is the only positive insertion expressed following a 2

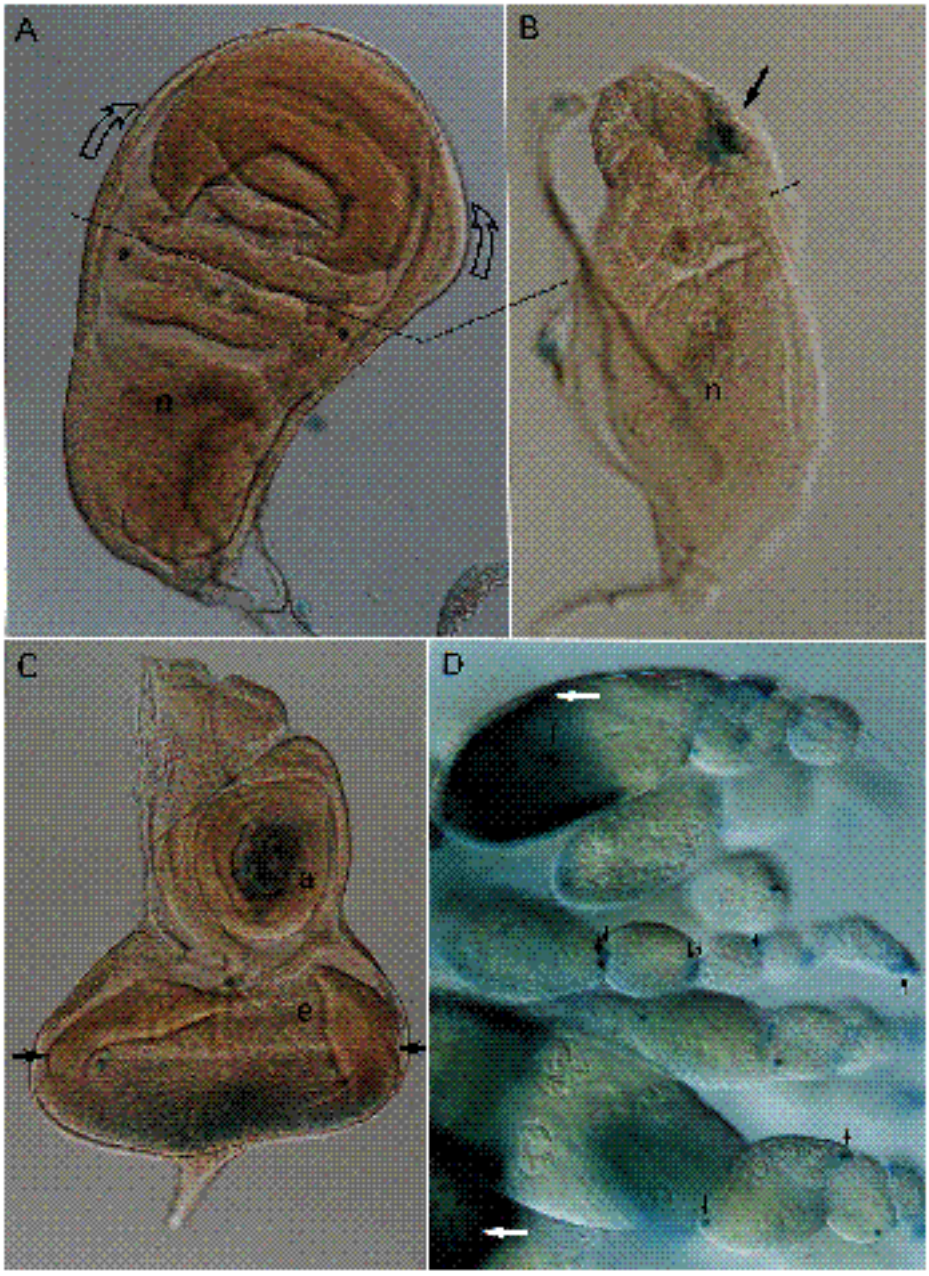

Fig. 5. β-galactosidase expression patterns in C-07. (A) Intact X-gal stained C-07 wing disc showing approximate position of cut (dashed lines) generating a notum fragment (n) for in vivo culture. (B) C-07 notum fragment stained after culture in adult female host. *lac-z* expression is localized (arrow) at presumed site of wound healing. Open arrows in A show folding of cut edge which would juxtapose normally non-expressing cells (near the dots) at the site of expression evident in B (arrow). (C) Untreated X-gal stained C-07 eye-antenna disc showing control expression in distal antenna (a) and and in eye disc (e) posterior to the morphogenetic furrow (arrows), where assembly of ommatidia is visible. (D) C-07 expression in adult ovary. X-gal staining is evident in the germarium (upward pointing arrow), in polar follicle cells (downward pointing arrows), and also in columnar follicle cells (white arrows) at late stages of oogenesis.

hour heat-shock at 37°C. It may detect a regulatory element activated in response to diverse sources of cellular stress, and also independently during regeneration. Remarkably, all seven of the compartment boundary lines tested were expressed ectopically in cultured disc fragments.

Differences in the site of ectopic expression can be seen among the examples shown in Fig. 4. In nineteen lines (listed in Table 3) ectopic expression was confined to the immediate neighbourhood of the wound heal (Fig. 4B-E, G-J, arrows); the other four lines (Table 3) also express *lac-z* in some cells remote from the wound. The precise pattern of expression at the site of wound healing apparently differs from line to line, e.g. in E-32 and F-36 (Fig. 4D,G) expression is clearly restricted to one side of the wound heal, implying a positional constraint on the activation of these insertions. Similarly, in AD-55 and other A/P boundary lines, ectopic expression has so far only been seen when the cells juxtaposed by wound healing are from different compartments; compare the standard a-a′ fragment in Fig. 4E (ectopic expression at site of A:P healing) with the posterior c-c′ fragment in Fig. 4F (P:P healing, no expression). This dependence implies that the cells confronted must monitor the compartmental states of their neighbours across the wound heal.

Expression patterns at other developmental stages

The insertions expressed in surgically fragmented discs were also examined for *lac-z* expression in oogenesis and during development of the embryo (Table 3). Two of the fifteen insertions with no control disc expression (E-37; F-45), were also not detectably expressed during normal

Table 2. Incidence of ectopic *lac-z* expression in disc fragments cultured in vivo*

control *lac-z* staining pattern	class of insertion			
	class I	class II	class III	class IV
no expression in control discs	2/5	13/49	1/6	0/14
compartment boundary expression in control discs	2/2	5/5	–	–
Total	4/7	18/54	1/6	0/14

*(Strains with ectopic expression in cultured fragments / total strains tested) is indicated for each category.

oogenesis or in the embryo. A further six lines (B-17, C-39, C-92, E-17, E-91, G-45) were only detectably expressed in embryonic CNS or PNS and three were also not expressed in oogenesis, ruling out a maternal contribution to normal disc development. Five of the lines were expressed at some stage of embryogenesis in patterns including the anlagen of the imaginal discs (D-42, E-34, E-60, F-22, H-21). The six insertions expressed along the A/P boundary showed five distinct combinations of adult ovary and embryonic *lac-z* expression.

More than half of the twenty-three insertions expressed in cultured disc fragments also showed *lac-z* expression in the adult ovary (Table 3). Nine lines stained in somatic follicle cells or germarium, and three in nurse cells. Several lines (C-76, E-60, C-07, H-87, AD-55) stained in a subset of follicle cells, e.g. in polar follicle cells as shown for C-07 in Fig. 5. As the figure shows, this line is also expressed posterior to the furrow in the eye disc, but only after injury, at the wound heal, in wing discs. It is notable that no less than five of the seven compartment boundary lines expressed in the regeneration blastema in cut discs also showed expression in the germarium or follicle cells.

DISCUSSION

Using an enhancer-sensitive P-element construct, we have conducted a systematic screen for genes expressed during imaginal disc regeneration. This is a well studied system, potentially important for elucidating the nature of positional information and its role in pattern formation (Wolpert, 1969), but one in which a genetic approach has not been systematically applied, due to the difficulty of screening by standard methods for mutations defective in the process. Because it identifies genes on the basis of their regulation, the new enhancer-trap technology presented an opportunity to search directly for genes with altered expression in regenerating discs. Since expression is detectable as a dominant phenotype, the insertions could be screened as heterozygotes over a wild-type chromosome to complement any essential functions which might be disrupted by an insertion. Thus we could identify a sample of loci unbiased with respect to their possible functions at earlier stages of normal development. This is not the case in an ordinary phenotypic screen.

Design and validation of screens

The initial screen made use of a temperature-sensitive cell-lethal mutation to induce disc lesions subsequently repaired by regeneration. We screened for altered *lac-z* expression

Table 3. *Lac-z* expression profiles of insertions ectopically activated in cultured wing disc fragments

Strain	wing disc staining		ovary pattern [stage]†	embryonic pattern
	control	fragment*		
B-17	none	2/7 at wound-heal	NC [10]	CNS
C-39	none	3/5 at wound-heal	NC [10]	CNS/PNS
C-92	none	8/19 at wound-heal	none	midline CNS (3 cells/segment)
D-42	none	6/9 at wound-heal	none	lateral epidermis (SEG)
E-17	none	5/11 at wound-heal	germarium	CNS/PNS
E-34	none	6/14 at wound-heal	none	weak
E-91	none	6/11 at wound-heal	none	CF, PNS
F-22	none	2/9 at wound-heal	none	weak
F-45	none	4/7 at wound-heal	none	none
G-45	none	7/16 at wound-heal	none	midline CNS (2 cells/segment)
H-21	none	5/16 at wound-heal	NC [10]	CF, epidermis (SEG)
C-76	none	7/10 general	FC, columnar [10]	midgut, Malphigian tubules
E-37	none	9/21 general	none	none
E-60	none	6/13 general	FC, posterior columnar [8]	weak
F-36	none	4/7 general	none	CF, dorsal epidermis
C-07	none‡	4/7 at wound-heal	FC,polar [1],columnar [9]	CNS
B-82	d-v margin	3/7 at wound-heal	FC [9]	ventral epidermis, PNS, hindgut
D-46	a/p boundary	6/13 at wound-heal	none	none
E-32	a/p boundary	12/21 at wound-heal	germarium, NC	lateral epid.(SEG),dorsal epid.
H-39	a/p boundary	9/16 at wound-heal	FC [12]	neurogenic epidermis
H-44	a/p boundary	4/11 at wound-heal	none	none
H-87	a/p boundary	5/11 at wound-heal	FC, squamous [9]	amnioserosa, dorsal epid.(SEG)
AD-55	a/p boundary	11/14 at wound-heal	FC, polar [8], border cells	mesodermal

*Number of fragments with *lac-z* expression / total number recovered; 'general' means ectopic staining away from the wound.

†Numbers in brackets refer to first stage (King, 1970) when expression is detected.

‡C-07 is expressed posterior to the morphogenetic furrow in eye disc, and in the tibia/AIII regions of the leg and antenna discs.

Abbreviations: NC: nurse cells; FC: follicle cells; CNS: part or all of central nervous system; PNS: part or all of peripheral nervous system; CF: cephalic furrow; SEG: segmental epidermal expression; epid.: epidermis.

24 hours after the end of the heat treatment, following cell death, and just prior to initiation of cell division in the regeneration blastema. This time point was chosen to coincide with early steps in regeneration that are important for specification of the new pattern (Girton and Russell, 1980, 1981). To eliminate false positives we re-screened during the heat treatment when the disc cells are just beginning to die, and therefore prior to initiation of regeneration. As a further tactic we reasoned that γ-radiation might induce a different set of cell-death genes (reviewed by Raff, 1992), but the resulting disc lesions would be repaired by a common regeneration mechanism. Application of these secondary screens gave us the four classes of enhancer-trap insertions shown in Fig. 3.

The effectiveness of the screens was generally confirmed by the results of in vivo culture experiments with disc fragments. More than half of the Class I insertions tested were ectopically expressed, and all but one of the remaining positives were from Class II. The substantial frequency of about one third of Class II insertions scored as positive in cultured disc fragments suggests that insertions in some regeneration genes may fail to respond to radiation-induced cell death under the conditions of our screen. Both cell-lethal and γ-ray induced lesions are thought to be repaired by intercalary cell divisions, but the early wound healing processes might be different because of the more uniform spatial distribution of the cell death in irradiated discs (Spreij, 1971). The cell lethal mutation (Clark and Russell, 1977) causes a strongly clustered pattern of cell-death (e.g. Fig. 1B). Also, because of the different time point at which the discs were assayed in the cell-lethal and γ-radiation screens, some transiently expressed functions may also have been detected only as Class II insertions.

The most persuasive evidence that the loci detected in our screens may be involved in regeneration comes from the expression patterns observed in disc fragments cultured in adult female hosts, where the cellular basis of the process has been best studied. In the positive lines from this test, ectopic expression was consistently localized in small numbers of cells at epithelial discontinuities. That these represent sites of wound healing, where the regeneration blastema originates (Bryant and Fraser, 1988), is indicated by the geometry of the folding of the disc epithelium that results in closure of the wound (Reinhardt et al., 1977; Reinhardt and Bryant, 1981; Dale and Bownes, 1985). The time at which expression was assayed also corresponds with recruitment of cells into the blastema as determined by clonal analysis (Girton and Russell, 1980, 1981). Our results therefore indicate extensive novel gene expression at the expected time and putative site of pattern regulation. We found differences among the lines in the extent and pattern of expression in the vicinity of the wound heal. This may reflect real diversity in the roles of the genes in wound healing or establishment of the blastema, but detailed studies will be required to define fully the temporal and spatial expression patterns characteristic of specific insertions.

Regeneration models and gene expression patterns selected by the screen

Although null mutant phenotypes will be necessary to assign definitive functions to individual genes, the preferential recovery in the screen of particular expression classes may provide some useful preliminary insights. Wound healing initiates regeneration by bringing into contact cells from different locations. This is thought to stimulate cell proliferation and the interpolation of intermediate positional values in the regeneration blastema (French et al., 1976). This model implies local cell communication at the site of wound healing, and control of the cell cycle, by positional signals from neighbours.

Wound healing

Morphological evidence has been obtained during wound healing for novel disc-cell behaviors such as motility, extension of pseudopodia and establishment of new contacts (Reinhardt et al., 1977; Reinhardt and Bryant, 1981). About a third of the insertions expressed in discs only after wounding define functions expressed normally in the embryonic CNS and PNS (Table 3). Other insertions with putative neurogenic primary functions are those expressed in the eye disc posterior to the morphogenetic furrow. This is where assembly of ommatidia occurs, a process involving cell rearrangements and formation of specific contacts, cell-communication leading to cell determination, and some cell death (Tomlinson, 1985; Cagan and Ready, 1989). The incidence of this expression pattern was specifically enhanced in the screen (Table 1). We speculate that neurogenic functions may be recruited in regeneration to mediate wound healing.

Establishment of field polarity

Our selected insertions are enriched for spatially patterned expression in subsets of follicle cells (26% as compared to 9% among the unselected insertions of Grossniklaus et al., 1989) and five out of seven ectopically expressed compartment boundary lines are also expressed in either follicle cells or germarium. Spatially patterned gene expression in the follicle cells helps determine the polarity of the embryo (Manseau and Schupbach, 1989; Ruohola et al., 1991) so the present results suggest the intriguing possibility of common steps in pathways for polarity specification in the regeneration blastema and in oogenesis. Other insertions are expressed in the embryonic germ band, which includes the precursors of the adult epidermal cell-lineage. For example, D-42 is expressed in a segmental repeat pattern in the lateral epidermis which could indicate a role in compartmental subdivision of embryonic segments.

Positional specification and compartment boundaries

15 of the 16 compartment boundary lines in the entire sample of 826 insertions were found in either class I or II and all seven lines tested were expressed ectopically in disc fragments. This may be significant in relation to the idea that compartment boundaries act as local organizers for the global specification of positional values (Meinhardt, 1983; Gelbart, 1989). The simple geometry of compartmentalization (Garcia-Bellido et al., 1973) would lend itself to specification of an orthogonal system of spatial coordinates, and experimental support for the cooperative involvement of different compartments in distal regeneration has been obtained (Schubiger and Schubiger, 1978; Karlsson, 1980). Genes expressed in compartmentally restricted patterns

could provide the initial spatial cues for respecification of the pattern.

The insertion in B-82, a compartment boundary line ectopically expressed in disc fragments, caused a lethal mutation which was revertible by excision of the P-element and allelic to *crumbs* (data not shown). *crumbs* is an EGF-repeat gene (Tepass et al., 1990). Its embryonic lethal phenotype suggests it may be necessary for maintaining the integrity of epithelia, but its expression in the wing disc has been reported as uniform (Tepass and Knust, 1990).

E-32, an ectopically expressed A/P boundary line, was found to be inserted at 22F, the cytological location of the *decapentaplegic* gene *(dpp)*. Characterization of flanking genomic DNA obtained by plasmid rescue has indicated that E-32 is inserted immediately adjacent to *dpp*, just downstream of the 3′ disc enhancer (R. K. Blackman, personal communication). Mutations in the disc region of *dpp* affect expression and cause distal pattern deletions in imaginal discs (Blackman et al., 1991) and the gene encodes a homologue of a vertebrate growth factor, TGF-β (Padgett et al., 1987). As an extracellular diffusible factor, the *dpp* product could be involved in the cell signaling postulated in the model of French et al. (1976), for mitogenic stimulation and specification of new positional values in the regeneration blastema. In this context it is interesting that expression of E-32 seems to be restricted to cells on one side of the wound heal in cultured disc fragments (Fig. 4D). That cell-cell interactions are involved in the regulation of gene activity in the regeneration blastema is also shown by the conditional expression of other A/P border insertions, which are apparently only ectopically expressed when cells from different compartments come together at a wound heal (e.g. AD-55, Fig. 4E and F). Although they are expressed in very similar patterns in the imaginal discs, the six insertions in the A/P boundary expression class probably have diverse functions, for they are expressed in various patterns at earlier stages of development (Table 3). It will be important to obtain mutations in the genes detected by these insertions and ascertain their epistatic relationships to see if they constitute a regulatory hierarchy.

It is remarkable that the formal rules devised to account for regeneration in insect epithelial systems also appear to work in the regenerating vertebrate limb (French et al., 1976; reviewed by Stocum, 1991). It was recently shown that certain of the HOX-complex genes, highly conserved vertebrate homologues of the *Drosophila* homeotic selector gene complexes, are spatially regulated by positional signals in the developing limb bud (Izpisua-Belmonte, et al., 1991) and a homeobox gene has been found to be redeployed in regenerating amphibian limbs (Brown and Brockes, 1991). In view of this it will be important to follow up the present studies with molecular and mutational analysis of the loci we have identified. The enhancer trap constructs have been designed to facilitate further investigation along these lines.

We thank Drs William Addison and Stanley Tiong for many useful discussions, Dr. David Pilgrim for use of a photomicroscope, Drs John Merriam, Ulrich Tepass and Yash Hiromi for fly stocks and plasmids and Dr Ron Blackman for cloning E-32 and permission to quote his unpublished results. W. J. B was supported by post-graduate scholarships from Natural Sciences and Engineering Research Council and the Alberta Heritage Foundation for Medical Research. J. P. and M. D. W. received undergraduate awards from the AHFMR and NSERC. This project was funded by NSERC grant no. OGP6485 to M. A. R.

REFERENCES

Abbott, L. C., Karpen, G. H. and Schubiger, G. (1981). Compartmental restrictions and blastema formation during pattern regulation in *Drosophila* imaginal leg discs. *Dev. Biol.* **87**, 64-75.

Baker, N. E. (1988). Transcription of the segment polarity gene *wingless* in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal *wg* mutation. *Development* **102**, 489-497.

Blackman,R.K.,Sanicola,M.,Raftery,L. A.,Gillevet,T.and Gelbart, W. M. (1991). An extensive 3′ cis-regulatory region directs the imaginal disc expression of *decapentaplegic*, a member of the TGF-β family in *Drosophila. Development* **111**, 657-665.

Bellen,H.J.,O'Kane,C.J.,Wilson,C.,Grossniklaus,U.,Pearson,R.K. and Gehring, W. J. (1989). P-element mediated enhancer detection: a versatile method to study development in *Drosophila. Genes Dev.* **3**, 1288-1300.

Brown, R. and Brockes, J. P. (1991). Identification and expression of a regeneration-specific homeobox gene in the newt limb blastema. *Development* **111**, 489-496.

Bryant,P.J. (1971). Regeneration and duplication following operations in situ on the imaginal discs of *Drosophila melanogaster*. *Dev. Biol.* **26**, 637-651.

Bryant, P. J. (1975). Pattern formation in the imaginal wing disc of *Drosophila melanogaster*: fate map, regeneration and duplication. *J. exp. Zool.*, **193**, 49-77.

Bryant, P. J. and Fraser, S. E. (1988). Wound healing, cell communication, and DNA synthesis during imaginal disc regeneration. *Dev. Biol.* **127**, 197-208.

Bryant, S. V., French, V., and Bryant, P. J. (1981). Distal regeneration and symmetry. *Science*, **212**, 993-1002.

Cagan, R. L. and Ready, D. F. (1989). The emergence of order in the *Drosophila* pupal retina. *Dev. Biol.* **136**, 346-362.

Clark,W.C. (1976). Histological investigations of a temperature-sensitive cell-lethal mutant of *Drosophila melanogaster*. M. Sc. Thesis. University of Alberta, Edmonton, Alberta, Canada.

Clark, W. C. and Russell, M. A. (1977). The correlation of lysosomal activity and adult phenotype in a cell-lethal mutant of *Drosophila*. *Dev. Biol.* **57**, 160-173.

Cooley,L.,Kelly,R.and Spradling,A. (1988). Insertional mutagenesis of the *Drosophila* genome with single P-elements. *Science* **239**, 1121-1128.

Dale,L.and Bownes,M. (1980). Is regeneration in *Drosophila* the result of epimorphic regulation? *Wilhelm Roux's Arch. dev. Biol.* **189**, 91-96.

Dale, L. and Bownes, M. (1985). Pattern regulation in fragments of *Drosophila* wing discs which show variable wound healing. *J. Embryol. exp. Morph.* **85**, 95-109.

Eaton, S. and Kornberg, T. B. (1990). Repression of *ci-D* in posterior compartments of *Drosophila* by *engrailed. Genes Dev.* **4**, 1068-1077.

French, V., Bryant, P. J. and Bryant, S. V. (1976). Pattern regulation in epimorphic fields. *Science* **193**, 969-981.

Garcia-Bellido, A., Ripoll, P. and Morata, G. (1973). Developmental compartmentalization of the wing disc of *Drosophila. Nature* **245**, 251-253.

Gelbart, W. M. (1989). The *decapentaplegic* gene: a TGF-β homologue controlling pattern formation in *Drosophila*. *Development* **Supplement** 65-74.

Girton, J. R. and Kumor, A. L. (1985). The role of cell death in the induction of pattern abnormalities in a cell-lethal mutation of *Drosophila*. *Dev. Genet.* **5**, 93-102.

Girton, J. R. and Russell, M. A. (1980). A clonal analysis of pattern duplication in a temperature-sensitive cell-lethal mutant of *Drosophila melanogaster*. *Dev. Biol.* **77**, 1-21.

Girton, J. R. and Russell, M. A. (1981). An analysis of compartmentalization in pattern duplications induced a temperature-sensitive cell-lethal mutation of *Drosophila melanogaster*. *Dev. Biol.* **85**, 55-64.

Grossniklaus,U.,Bellen,H.J.,Wilson,C.and Gehring,W.J. (1989). P-element-mediated enhancer detection applied to the study of oogenesis in *Drosophila*. *Development* **107**, 189-200.

Hama, C., Ali, Z., and Kornberg, T. B. (1990). Region specific recombination and expression are directed by portions of the Drosophila engrailed promoter. *Genes Dev.* **4**, 1079-1093.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.

Izpisua-Belmonte,J.C.,Tickle,C.,Dolle,P.,Wolpert,L.and Duboule, D. (1991). Expression of the homeobox Hox-4 genes and the specification of position in chick wing development. *Nature* **350**, 585-589.

Jacobs, J. R., Hiromi, Y., Patel, N. H. and Goodman, C. S. (1989). Lineage, migration, and morphogenesis in the *Drosophila* CNS as revealed by a molecular lineage marker. *Neuron* **2**, 1625-1631.

James, A. A. and Bryant, P. J. (1981).A quantitative study of cell death and mitotic inhibition in γ-irradiated imaginal wing discs of *Drosophila melanogaster*. *Radiat. Res.* **87**, 552-564.

Karlsson,J. (1980). Distal regeneration in proximal fragments of the wing disc of *Drosophila*. *J. Embryol. exp. Morph.* **59**, 315-323.

King, R. C. (1970). *Ovarian Development in Drosophila melanogaster*. New York and London: Academic Press.

Kornberg, T., Siden, I., O'Farrell, P. and Simon, M. (1985). The *engrailed* locus of *Drosophila*: In situ localization of transcripts reveals compartment-specific expression. *Cell* **40**, 45-53.

Lindsley, D. L. and Zimm, G. G. (1992). *The genome of* Drosophila melanogaster. San Diego, USA: Academic Press.

Manseau,L. J.and Schupbach,T. (1989). The egg came first, of course! *Trends Genet.* **5**, 400-405.

Martinez-Arias, A. (1989). A cellular basis for pattern formation in the insect epidermis. *Trends Genet.* **5**, 262-267.

Mazo, A. M., Mizrokhi, L. J., Karavanov, A. A., Sedkov, Yu. A., Krichevskaya, A. A. and Ilyin, Yu. V. (1989). Suppression in *Drosophila*: *su(Hw)* and *su(f)* gene products interact with a region of *gypsy (mdg4)* regulating its transcriptional activity. *EMBO J.* **8**, 903-912.

Meinhardt,H. (1983). Cell determination boundaries as organizing regions for secondary embryonic fields. *Dev. Biol.* **96**, 375-385.

Nash,D.and Bell,J.B. (1968). Larval age and pattern of DNA synthesis in polytene chromosomes. *Can J.Genet. Cytol*. **15**, 237-254.

O'Kane, C. J. and Gehring, W. J. (1987). Detection in situ of genomic regulatory elements. *Proc. natl. Acad. Sci. USA* **84**, 9123-9127.

Padgett, R. W., St. Johnston, R. D. and Gelbart, W. M. (1987). A transcript from a *Drosophila* pattern gene predicts a protein homologous to the transforming growth factor-β. *Nature* **325**, 81-84.

Pattattucci, A. M. and Kaufman, T. C. (1991). The homeotic gene *Sex combs reduced* of *Drosophila melanogaster* is differentially regulated in the embryonic and imaginal stages of development. *Genetics* **129**, 443-461.

Phillips, R. G., Roberts, I. J. H., Ingham, P. W. and Whittle, J. R. S. (1990). The *Drosophila* segment polarity gene *patched* is involved in a position-signalling mechanism in imaginal discs. *Development* **110**, 105-114.

Postlethwaite, J. H. and Schneiderman, H. A. (1973). Pattern formation and determination in imaginal discs of *Drosophila melanogaster* after irradiation of embryos and young larvae. *Dev. Biol.* **32**, 345-360.

Raff, M.C. (1992). Social controls on cell survival and cell death. *Nature* **356**, 397-400.

Reinhardt,C.A.and Bryant,P.J. (1981). Wound healing in the imaginal discs of *Drosophila* II. Transmission electron microscopy of normal and healing wing discs. *J. exp. Zool.* **216**, 45-61.

Reinhardt, C. A., Hodgkin, N. M. and Bryant, P. J. (1977). Wound healing in the imaginal discs of *Drosophila* I. Scanning electron microscopy of normal and healing wing discs. *Dev. Biol.* **60**, 238-257.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D., Benz, W. K. and Engels, W. R. (1988). A stable source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**, 461-470.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. and Jan, Y.N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 433-449.

Russell, M. A. (1974). Pattern formation in the imaginal discs of a temperature sensitive cell lethal mutant of *Drosophila melanogaster*. *Dev. Biol.* **40**, 24-39.

Russell,M.A. (1985). Positional information in insect segments. *Dev. Biol.* **108**, 269-283.

Schubiger,G. (1971). Regeneration, duplication and transdetermination in fragments of the leg disc of *Drosophila melanogaster*. *Dev. Biol.* **26**, 277-295.

Schubiger, G. and Schubiger, M. (1978). Distal transformation in *Drosophila* leg imaginal disc fragments. *Dev. Biol.* **67,** 286-295.

Simcox,A.A.,Roberts,I.J.H.,Hersperger,E.,Gribbin,M.C.,Shearn, A. and Whittle, J. R. S. (1989). Imaginal discs can be recovered from embryos mutant for the segment polarity genes *engrailed*, *naked*, and *patched* but not from *wingless. Development* **107**, 715-722.

Spreij, T. E. (1971). Cell death during the development of the imaginal discs of *Calliphora erythrocephala. Netherlands J. Zool.* **21**, 221-264.

Stocum,D.L. (1991). Limb regeneration: a call to arms (and legs). *Cell* **67**, 5-8.

Szabad, J., Simpson, P. and Nöthiger, R. (1979). Regeneration and compartments in *Drosophila. J. Embryol. exp. Morph*. **49**, 229-241.

Tepass, U. and Knust, E. (1990). Phenotypic and developmental analysis of mutations at the *crumbs* locus, a gene required for the development of epithelia in *Drosophila melanogaster*. *Wilhelm Roux's Arch. dev. Biol.* **199**, 189-206.

Tepass,U.,Theres,C.and Knust,E. (1990). The *Drosophila* gene *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for the organization of epithelia. *Cell* **61**, 787-799.

Tomlinson,A. (1985). The cellular dynamics of pattern formation in the eye of *Drosophila*. *J. Embryol. exp.Morph*. **89**, 313-331.

Vincent, J.-P., and O'Farrell, P. H. (1992). The state of *engrailed* expression is not clonally transmitted during early *Drosophila* development. *Cell* **68**, 923-931.

Wilkins, A.S. and Gubb,D. (1991). Pattern formation in the embryo and imaginal discs of *Drosophila*: what are the links? *Dev. Biol.* **145**, 1-12.

Wolpert,L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1-47.

(Accepted 24 November 1992)