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Identifying loci required for follicular patterning using directed mosaics

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SUMMARY

We have developed a 'directed mosaic' system in *Drosophila* by using the GAL4 system to control the expression of the yeast recombinase, FLP, in a spatial and temporal fashion. By directing FLP expression, we show that it is possible to efficiently and specifically target loss-of-function studies for vital loci to the developmental pathway of interest. A simple F_1 adult phenotypic screen demonstrated that most adult tissues can be analyzed with this approach. Using GAL4 lines expressed during oogenesis, we have refined the system to examine the roles of vital loci in the development of the follicular epithelium. We have identified essential genes involved in egg chamber organization, cell migration and cell shape. Further, we have used this technique to gain insights into the role of the *Drosophila* EGF receptor pathway in establishing the egg axes. Finally, using different UAS-FLP, GAL4 and existing FRT lines, we have built stocks that permit the analysis of ~95% of the genome in follicular mosaics.

Key words: *Drosophila*, mosaics, UAS-FLP, follicle cells, oogenesis

INTRODUCTION

From a single cell with finite resources, the process of development calls forth an astonishing array of cellular diversity. How is such a task accomplished? The use of a single molecule in multiple developmental processes provides a simple means to create diversity from limited resources. Recently, discoveries that particular proteins or entire signaling pathways are used in multiple biological processes have become increasingly common. As a result, this concept of molecular reutilization has moved to the forefront of developmental biology. Experimentally, this presents significant technical difficulties. The activity of a molecule in one developmental pathway can obscure its role(s) in other developmental processes. This knowledge has necessitated the generation of more sophisticated methods with which to address the role of such proteins and pathways in diverse biological processes (Perrimon and Gans, 1983; Golic and Lindquist, 1989; Golic, 1991; Chou and Perrimon, 1992; Brand and Perrimon, 1993; Chou et al., 1993; Xu and Rubin, 1993; Struhl and Basler, 1993; Calleja et al., 1996; Rørth, 1996).

Historically, biologists have relied upon the ability to create mosaics, organisms in which the genotype varies in a cell- or tissue-specific manner, to circumvent such problems. In organisms where genetic analysis is feasible, the induction of mitotic recombination has been used to produce mosaics. When a cell heterozygous for a particular mutation undergoes mitotic recombination, the resulting chromosomal exchange coupled with assortment and division can result in homozygosity for one of the progenitors. Classically, mitotic recombination has been induced by X-irradiation. However, X-irradiation results in a low frequency of mosaicism and high frequency of cell death. These technical problems have been circumvented in *Drosophila melanogaster* using the yeast site-specific recombinase, FLP (Golic and Lindquist, 1989; Golic, 1991; Chou and Perrimon, 1992; Xu and Rubin, 1993). FLP catalyzes recombination at sequence motifs termed FRTs. Integration of FRTs in a *cis* or *trans* configuration within the genome provides a direct means to initiate site-specific mitotic recombination. As such, induction of FLP expression via a ubiquitous (heat-shock, hs) promoter is capable of significant increases in the frequency of mosaicism without the associated lethality of X-irradiation. However, as with X-irradiation, this approach is still limiting. Ubiquitous induction of FLP via heat shock provides only a limited degree of spatial and temporal control. While the use of heat shock can direct mosaic patches that are widely distributed throughout the animal, it typically does not result in the population of any one tissue with all or mostly mutant tissue. Furthermore, the induction of mosaic patches by heat-shock-directed FLP may cause lethality by virtue of the extensive amount of tissues that are made mosaic.

By selectively targeting FLP activity to specific cells or tissues using the GAL4 system (Brand and Perrimon, 1993), we have developed a genetic system that overcomes these limitations. With this approach, the loss of gene activity can be directed to any process of interest. As we document below, the 'directed mosaic' approach provides an efficient way to bypass the vital requirements of lethal mutations and assay

their effect on specific tissues during later stages of development.

Previous work has suggested that establishment of the body axes in *Drosophila melanogaster* requires cellular communication between the germline-derived oocyte and its overlying somatic-derived follicular epithelium (Ray and Schupbach, 1996). The germline contribution of any gene to these processes can be examined through hs-induced mosaics and selected for using the dominant-female sterile mutation, *ovo*^{D1} (Chou and Perrimon, 1992). In contrast, no such directed or selective system exists for the analysis of gene requirements within the follicular epithelium. To identify vital loci required for the somatic components of this intercellular signaling and to complement the germline mosaic system, we focused our efforts with the directed mosaic system on the follicular epithelium. Using two different assays for follicular clones, we first document the parameters of the system and establish it as an efficient approach to control the production of mitotic clones in a temporal and spatial fashion. Next, vital loci whose effects on follicular development include alterations in A/P and D/V axis formation, egg chamber organization, cell migration and cell shape were identified using this approach. Support for a requirement of receptor tyrosine kinase (RTK) signaling within posterior follicle cells for axis formation and the specification of embryonic dorsal/ventral (D/V) pattern in part by limited diffusion of a ventralizing activity has also been provided. Finally, to extend the usefulness of this system, we have generated FLP/GAL4/FRT stocks that allow ~95% of the genome to be tested in follicular mosaics.

MATERIALS AND METHODS

Drosophila melanogaster strains

The *en-GAL4* and *e22c-GAL4* lines used in this study were generated in our laboratory and are described in Sanson et al. (1996) and Yoffe et al. (1996), respectively. The *lacZ* FLP-out cassette line is described in Struhl and Basler (1993). The *zeste-white3* allele, *zw3*^{M11}, was used for making wing clones and is described in Siegfried et al. (1992). The *T155-GAL4* line was provided by J. Urban and G. Technau. The *UAS-Green fluorescent protein* (*UAS-GFP*) is described in Yeh et al. (1995). The Ubiquitin-*GFP* (*Ub-GFP*) is described in Davis et al. (1995).

Construction of *UAS-FLP*

The *FLP* gene was excised from the vector pDM420 (kindly provided by K. Golic) as a *Sal*I (nt 5280) to *Xba*I (nt 6643) fragment. This fragment was then cloned into the *Xho*I and *Xba*I sites of the vector pUAST to generate pUAST-FLP (*UAS-FLP*). This construct was injected into *y*, *w*; Δ2-3, *Sb*/*TM6*, *Ubx* (Spradling, 1986; Robertson et al., 1988). From these injections, a homozygous viable second chromosome line was established. Using this line as a starter and Δ2-3-mediated transposition, a homozygous viable third chromosome line was generated.

T155-GAL4-directed mosaic screen for adult phenotypes

For the F_1 screen for adult phenotypes using *T155-GAL4*, the 2L FRT chromosomes used are described in Harrison and Perrimon (1993). Males carrying the 2L FRT (E24-4) and the *T155-GAL4* were fed 15 mM EMS in 1% sucrose for 12-20 hours, allowed to recover and then mated to females homozygous for the 2L FRT (E24-1) and a second chromosome *UAS-FLP* insert. From this cross, 6665 F_1 progeny were scored for adult abnormalities. Any F_1 adults with a phenotype (total = 835) were backcrossed to the homozygous 2L FRT (E24-1) and *UAS-FLP* line. 527 of these crosses gave rise to progeny. The F_2 from these crosses were scored for the presence of the appropriate phenotype (seen in the F_1) to determine its heritability and penetrance. Only 58% of these 527 lines bred true in the second generation. This is most likely a result of the mutagenic mechanism by EMS. A single EMS lesion results in the modification of only one DNA strand (Ashburner, 1989). As a result, mutational events that occur during meiosis may be seen in only half the cells of a resulting zygote. Consequently, the mutated DNA strand may not be represented in the germline of that mutant and thus will not be inherited by its offspring. Nevertheless, 58% of the mosaic animals in our screen had heritable lesions. The use of X-irradiation or other mutagens that affect both DNA strands should result in a higher proportion of lesions breeding true.

Follicle cell clone assays

The X chromosome *lacZ* enhancer-trap line *AN365* was provided by T. Schüpbach and is expressed in follicle cells. A recombinant X chromosome carrying AN365 and the FRT^{101} was provided by R. Hsu. For the second assay, three different recombinant lines containing the *tsl* allele *tsl*^{621} and the third chromosome FRT^{2A} were provided by A. Amon and R. Lehmann.

X chromosome lethal screen

For the X chromosome-directed mosaic screen, a subset (containing only germ cell viables) of lethals from Chou and Perrimon (1992) was used. Virgin females of the genotype: *y*, *w*, *lethal*, FRT^{101}/FM7 were mated to *w*, *AN365*, FRT^{101}/Y; *e22c-GAL4*, *UAS-FLP*/*CyO* males. F_1 virgin females of the genotype: *y*, *w*, *lethal*, FRT^{101}/*w*, *AN365*, FRT^{101}; *e22c-GAL4*, *UAS-FLP* were collected and mated to OreR males. From 2-4 days posteclosion, individual vials were examined for deposited eggs, which were then examined for defects. After this, the ovaries of directed mosaic females representing each lethal line were dissected, stained for *lacZ* activity and DAPI, mounted in 70% glycerol/PBS and examined for the presence of follicular clones and ovarian defects.

MEK, *Csw* and *GAP1* analyses

To mark *MEK*^{756} follicle cell clones with *GFP*, the X chromosome line Ub-*GFP*^{33C1} was recombined onto an X chromosome containing the FRT^{101}. Virgin females of the genotype: *y*, *w*, *MEK*^{756}, FRT^{101}/*FM7* ; *e22c-GAL4*, *UAS-FLP*/*CyO* were mated to *y*, *w*, Ub-*GFP*^{33C1}, FRT^{101}/Y. F_1 females were collected and ovaries were dissected from 1-6 days posteclosion. Ovaries were then processed for *GFP* fluorescence by fixation in 4% methanol-free formaldehyde diluted from a 16% solution with phosphate-buffered saline plus 0.1% Tween 20 or alternatively for in situ hybridizations with a digoxigenin-labelled antisense RNA probe for *bicoid* and mounted in 70% glycerol/PBS plus Slow-fade (Molecular Probes) (Gonzalez-Reyes et al., 1995). Directed mosaic females for *GAP1* were generated in the following manner. Virgin females of the genotype: *w*; *GAP1*^{B2}, FRT^{2A}/*TM3*, *Sb* were mated to *w*; *e22c-GAL4*, *UAS-FLP*/*CyO*; FRT^{2A}/*TM3, Sb* males. F_1 virgin females of the genotype: to *w*; *e22c-GAL4*, *UAS-FLP*/ + +; *GAP1*^{B2}, FRT^{2A}/FRT^{2A} were collected and mated to OreR males. Embryos were collected from these F_1-directed mosaic females and processed for cuticle preparations or immunohistochemistry (Kania et al., 1990; Duffy et al., 1996) using a digoxigenin-labelled antisense RNA probe for *twist* or a mouse monoclonal antibody to Dorsal. The α-Dorsal antibody was provided by R. Steward, used at dilution of 1/10, detected with fluorescein-labelled α-mouse IgG (Vector Labs) at a dilution of 1/300 and mounted in 70% glycerol/PBS plus Slow-fade (Molecular Probes).

RESULTS

Production of a GAL4-responsive FLP recombinase

To overcome the limitations of hs-induced FLP expression, we

took advantage of the GAL4 gene expression system (Brand and Perrimon, 1993). This binary gene activation system utilizes the yeast activator GAL4 to quantitatively and qualitatively control the transcription of target genes. By fusing the gene of interest to a GAL4-responsive promoter (UAS), its expression can then be directed with an appropriate GAL4 enhancer-trap line.

By building a GAL4-responsive FLP (*UAS-FLP*), we hoped to provide a high degree of spatial and temporal control over recombination. Fig. 1 documents such control. Using an *en-GAL4* line, we directed expression and activity of FLP to the posterior compartment of imaginal discs (Sanson et al., 1996). Fig. 1A documents this directed *cis* (intrachromosomal) recombination with β-galactosidase (*lacZ*) expression from a FLP-out cassette developed by Struhl and Basler (1993). Their cassette is organized in the following fashion: *actin5C* promoter-FRT-*Draf*$^{+}$ -FRT-*lacZ*. The combined presence of *en-GAL4* and *UAS-FLP* should result in *cis* recombination at the flanking FRTs, removal of the *Draf*$^{+}$ fragment and expression of *lacZ* due to its subsequent juxtaposition to the *actin5C* promoter. As expected, *lacZ* expression was observed and restricted to the posterior compartment indicating that FLP activity was spatially targeted by the *en-GAL4* driver (Fig. 1A). In addition, the presence of *UAS-FLP* and *en-GAL4* was required to observe *lacZ* expression indicating that both components are necessary for directed recombination (data not shown).

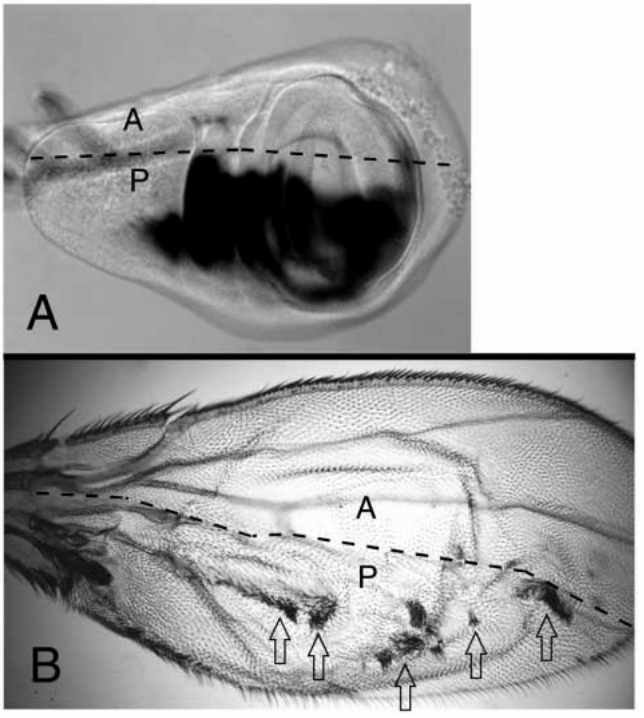


Fig. 1. Directed *cis* and *trans*-recombination in the wing imaginal disc mediated through *UAS-FLP* and *en-GAL-4*. (A) β-gal staining depicting *cis*-recombination with the Struhl and Basler FLP-out cassette. (B) *Trans*-recombination with *zw3* FRT^{101}. Clones of *zw3*^{M11} result in ectopic bristles in the wing. When directed with *en-GAL4* and *UAS-FLP*, these ectopic bristles are limited to the posterior compartment (marked by the outlined arrows). The compartments are marked with A, anterior; P, posterior. The compartment boundary is depicted with a hatched line.

Similarly, directed *trans* (interchromosomal) recombination was also demonstrated. Mutant clones of the segment-polarity gene *zw3* produced in the developing wing imaginal disc result in the production of ectopic bristles (Simpson et al., 1988). Mutant clones of *zw3* generated via *en-GAL4* and *UAS-FLP* resulted in the production of ectopic wing bristles only within the posterior compartment (Fig. 1B). Thus, directed *cis* and *trans* recombination can be obtained with the GAL4-responsive *UAS-FLP*.

GAL4-responsive FLP provides a directed method for F_1 mutant screens

Screening a series of random lethal mutations for phenotypes is a powerful genetic tool for dissecting developmental processes. However, the classical approach to recovering such mutations requires generating lethal mutations to be maintained as individual stocks before analysis. Such F_2 screens are both time consuming and labor intensive. A more efficient approach is to analyze the first generation of progeny directly for mutant phenotypes (an F_1 screen). Due to the recessive and/or lethal nature of most mutations, however, this is not possible without mosaic techniques. As described above, the lack of tissue specificity associated with previous methods for inducing mitotic clones may be a limitation in F_1 screens for lethal mutations. Restricting mutant clones to the tissue of interest should retain high viability of mosaic animals.

To address the feasibility of such a directed screen in adult tissues with the *UAS-FLP*, we carried out the following scheme. Males carrying a FRT inserted at the base of 2L and the *T155-GAL4* line were mutagenized with EMS. These males were then crossed to females also carrying the 2L FRT and the *UAS-FLP* construct. In the progeny of this cross, *T155-GAL4*-directed *UAS-FLP* expression results in adults mosaic for mutations induced on 2L. The GAL4 insert (T155) used in this screen directs GAL4 activity to the imaginal discs during mid and late larval stages. 6665 F_1 adults from this screen were scored for visible phenotypes and the results are summarized in Table 1. Over 12% (835) of the adults recovered in the screen had easily scored visible phenotypes. These phenotypes represented a broad spectrum of developmental abnormalities, ranging from eye defects to homeotic transformations. From this, we conclude that directed mosaicism provides a potent approach for genetic investigations of specific developmental processes.

Targeting mitotic recombination to the follicular epithelium with *UAS-FLP*

Having demonstrated the general feasibility of this system, we wanted to concentrate our efforts on the primary objective of this approach: adult tissue-specific screens for vital loci.

To complement the germline mosaic technique, we chose to

Table 1. Adult mosaic screen

Phenotypic class	No. of flies scored in phenotypic class/total no. of flies scored	Frequency
Eye defects	451/6665	6.8%
Wing defects	456/6665	6.8%
Leg defects	58/6665	0.9%
Bristle defects	27/6665	0.4%
Homeotic trans.	2/6665	0.03%
Extra sex combs	2/6665	0.03%
Other	63/6665	0.9%

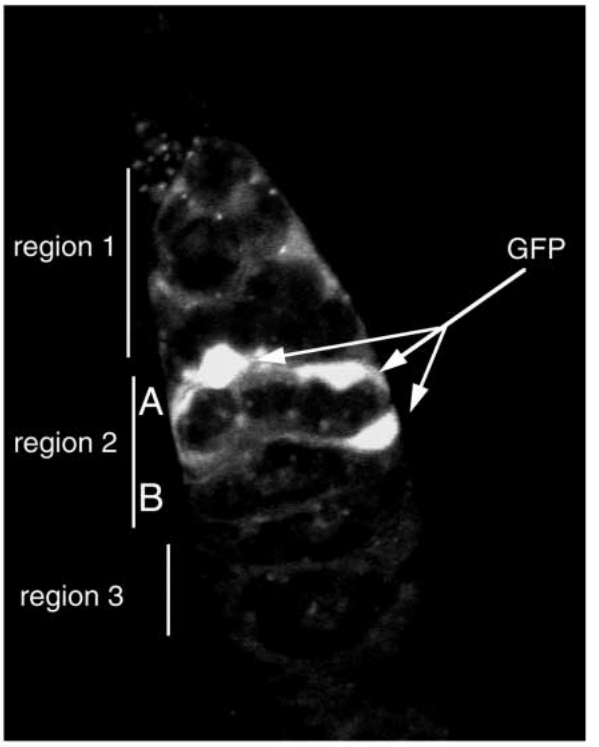
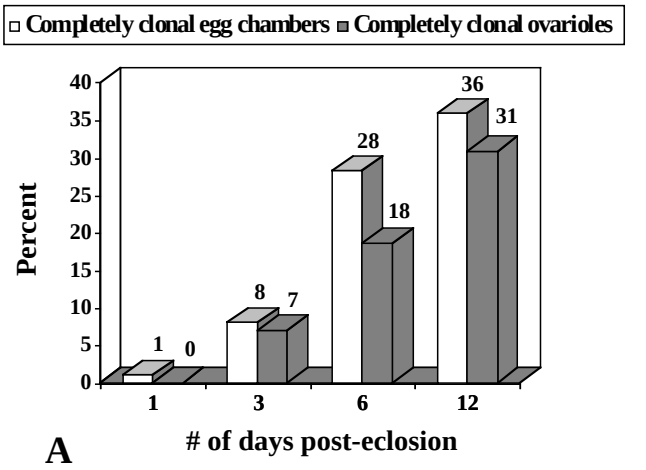


Fig. 2. Identification of a GAL4 line expressed in the presumptive follicular stem cells. A germarium dissected from a female carrying the *e22c-GAL4* and *UAS-GFP* lines is shown. The three subdivisions of the germarium are marked on the left. GFP fluorescence (marked with arrows) is seen in region 2 in the location of the follicular stem cells.



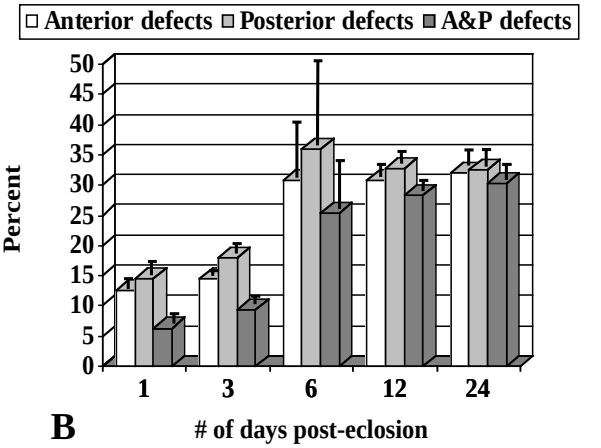


Fig. 3. (A) Temporal analysis of *e22c-GAL4*-directed follicular mosaics assayed with *AN365*/*lacZ*. Males carrying the X chromosome enhancer-trap line *AN365* with FRT^{101} and a recombinant second chromosome carrying both the *UAS-FLP* and the *e22c-GAL4* transposons were mated to females carrying the FRT^{101} insert. Virgin F_1 females were collected and outcrossed to OreR males. At 1, 3, 6 and 12 days posteclosion, these females were dissected and scored for the presence of any follicle cell clones by β-gal staining as described in Brand and Perrimon (1994). 136 egg chambers were scored for day 1, 197 for day 3, 553 for day 6 and 154 for day 12. The percentages of egg chambers or ovarioles that were composed of a completely clonal follicular epithelium (as marked by the absence on *lacZ* expression) are shown for the various time points. The actual percentages are indicated above their respective bars. (B) Temporal analysis of *e22c-GAL4*-directed follicular mosaics assayed with *tsl*. FRT^{2A}, tsl^{621} /*TM3, Sb* females were mated to *w*; *e22c-GAL4, UAS-FLP*/*CyO*; FRT^{2A}/*TM3, Sb* males to produce F_1 females with follicle cell clones. Virgin F_1 females were collected and mated to OreR males. At 1, 3, 6, 12 and 24 days posteclosion embryos were collected from these females and scored for their cuticular phenotypes according to the method described in Duffy et al. (1996). The percentage of embryos exhibiting anterior or posterior, or both types of defects was determined for each line, independently. Each bar represents the mean of the data collected from the three recombinants. Error bars indicate the standard deviation for each time point. A total of 602 embryos were scored for day 1; 1619 for day 3; 560 for day 6; 825 for day 12; and 433 for day 24. The percentages of embryos that had anterior or posterior, or both types of defects are shown at the various time points.

further develop this method to direct mutant clones to the follicular epithelium of the adult female. We screened for and identified GAL4 enhancer-trap lines that are somatically expressed in region 2A of the germarium, the location of the follicular stem cells (Margolis and Spradling, 1995), and not in the germline (Fig. 2, data not shown) using *UAS-LacZ*, *UAS-GFP* and *lacZ* FLP-out cassettes as reporters. Using one of these GAL4 lines, *e22c-GAL4*, the following questions were addressed: (1) can clones be directed to the follicular epithelium and (2) how efficient is clone production within the epithelium (what are the size and frequency of clones)?

To answer these questions, we used two viable assays. In the first assay, one of the FRT chromosomes was marked with an enhancer-trap line that is expressed within the follicular epithelium. This allowed the identification of follicular clones on the basis of *lacZ* staining. The results of this analysis are shown in Fig. 3. From these data, a number of points can be made. First, clones can be directed to the follicular epithelium. Second, since egg chambers and ovarioles whose entire follicular epithelium is monoclonal can be recovered, *e22c-GAL4* must be expressed in the follicular stem cells (or their precursors). Third, the frequency of monoclonal egg chambers and ovarioles changed as the females aged, reaching a maximum of 36% and 31%, respectively.

These results compare favorably with the results of Margolis and Spradling (1995), who observed an increase in monoclonal ovarioles from initially mosaic ovarioles induced via hsFLP. They

also conclude that there are 2-3 follicular stem cells per ovariole and that follicular stem cells have a finite lifespan. With *e22c-GAL4*-directed mosaics, we observed an increase in monoclonal ovarioles (either homozygous state) as the females aged. This strongly suggests that when FLP is driven by *e22c-GAL4*, the follicular stem cells were initially heterozygous and after eclosion FLP-mediated mitotic recombination occurred during subsequent rounds of stem cell division. The parent stem cell and its progenitor then adopted contrasting homozygous states. The conversion of the stem cell to a homozygous state, coupled with its finite lifespan resulted in the transformation of initially mosaic ovarioles to a monoclonal state as the females aged. This mechanism is also supported by our observation that none of the day 1 chambers (and thus ovarioles) scored were completely clonal, as might be predicted if conversion were taking place prior to the establishment of a stem cell population. Instead 99% of these day 1 egg chambers were mosaic (data not shown). Small clones were present in these day 1 egg chambers indicating that mitotic recombination must also occur within the follicular cells during S1-S6. Consistent with this, *e22c-GAL4* activity can be detected during these stages. Finally, no follicular clones were seen in control progeny lacking any one of the four components of the system (FRTs, *UAS-FLP*, *e22c-GAL4*) demonstrating that all elements are necessary for directing mosaics.

To further characterize the parameters of this system, a second assay employing a FRT chromosome bearing a mutation in the gene *torsolike* (*tsl*) was used. Tsl activity is required in the follicle cells for the establishment of the embryonic anterior and posterior termini (Stevens et al., 1990; Martin et al., 1993; Savant-Bhonsale and Montell, 1993). Loss of Tsl activity within the follicle cells results in the loss of these embryonic terminal structures. By scoring the cuticular phenotype of embryos derived from directed *tsl*$^{-}$ mosaic females, we could indirectly assay the frequency and size of mutant clones of *tsl* within the follicular epithelium (Fig. 3B). As shown in Fig. 3B, results similar to those seen in the *lacZ* assay were obtained. Moreover, the results with *tsl* clones also suggest that monoclonal frequencies reach a maximal level of ~30% around 6 days posteclosion. From these two assays, we conclude that GAL4-directed FLP-mediated mitotic recombination is an efficient approach to regulate the spatial and temporal induction of mosaics.

Revealing follicular requirements for vital loci

The results above indicate that mosaics can be generated within the follicular epithelium with a frequency and efficiency suitable for genetic screens. However, both of these assays involved the use of a non-lethal marker (*lacZ*) and mutation (*tsl*). To use the directed mosaic approach to identify vital loci required for follicular patterning, we must be able to obtain mosaic adults with clones of lethal mutations within the follicular epithelium. If *e22c-GAL4* (or any other GAL4 line used to make follicular mosaics) was expressed during earlier developmental stages, this could result in the production of clones in other tissues and lethality.

Therefore, we carried out a small-scale directed mosaic screen with *e22c-GAL4* as the clone driver (see Materials and Methods). A collection of lethal mutations from Chou and Perrimon (1992) induced on an X chromosome carrying FRT^{101}

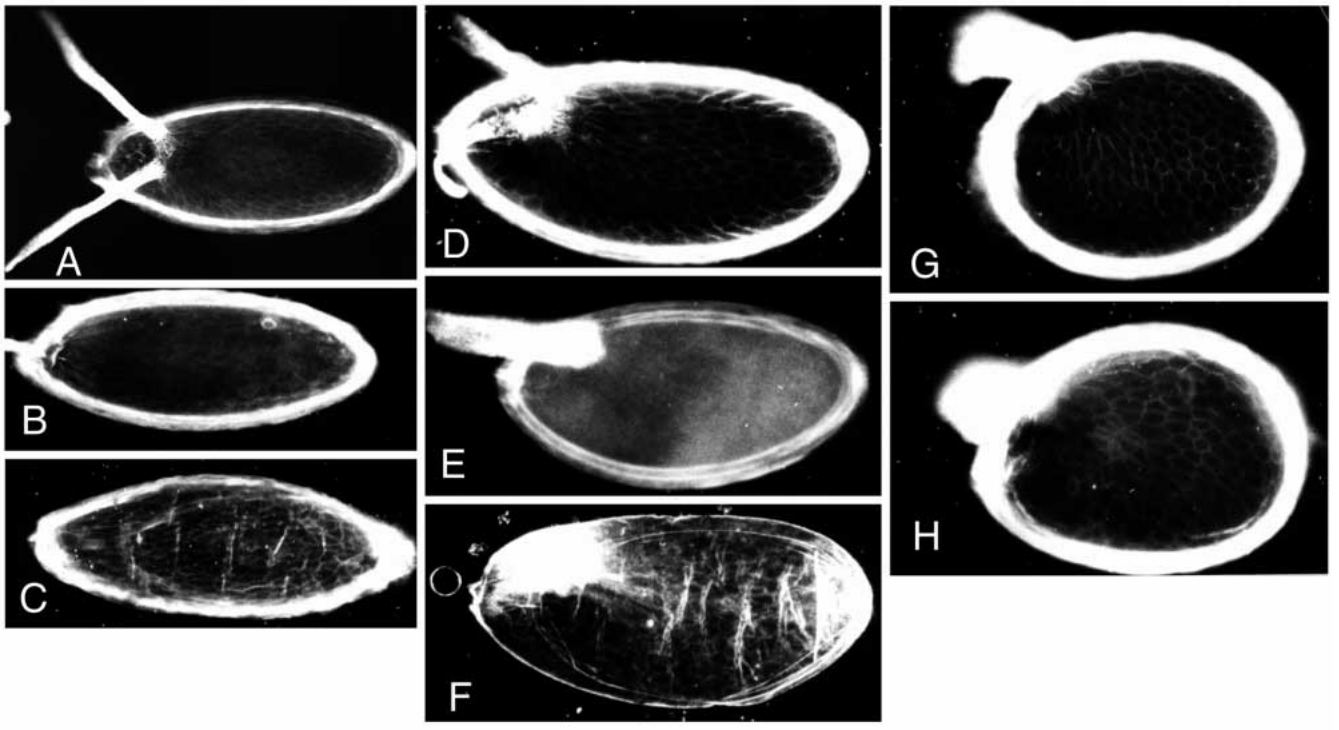


Fig. 4. Chorion phenotypes from X-chromosome follicular mosaics. Dark-field micrographs of chorions are shown in A-H. (A) A wild-type chorion; (B-H) chorions representative of the phenotypes observed from the directed screen with X chromosome lethals. (B,C) Ventralized chorions seen with follicle cell clones of *MEK*^{756} and *Csw*^{817}. (D-F) Mutant phenotypes from lines (611, map position 1-24.4; 131; and 671, respectively) that affect only D/V and A/P chorionic structures. (G,H) Mutant phenotypes from putative *mys* lines (968, map position 1-21 and 1070, map position 1-21.2) affecting the overall shape of the chorion and follicle cells.

(14A-B) was screened for: (1) the presence of mosaic adults, (2) follicular clones and (3) phenotypic defects. Out of the 93 lines carrying recessive lethal mutations, adult females of the appropriate genotype were recovered for all lines. Similar results were obtained with another GAL4 line (J. Duffy, unpublished). Of these 93 lines, 89 produced adult mosaic females with follicular clones. From these lines, a wide variety of defects, ranging from alterations in A/P and D/V polarity, egg chamber organization, cell migration and cell shape were observed (Figs 4, 5; data not shown). As described below, three of these lines, 667, 817, 756, correspond to previously identified components of RTK signaling. In addition, two lines, 968 and 1070, exhibiting defects in the overall shape of the chorion and follicular cells were genetically mapped to the region containing *myospheriod*, the β subunit of the position-specific integrins PS1 and PS2 (Fig. 4G,H). Consistent with the possibility that these lines have mutations in *mys*, FLP-induced imaginal clones of 968 and 1070 produced wing blisters similar to that seen with *mys* clones (Brower and Jaffe, 1989).

With the successful results for this X chromosome screen, we have now generated FLP/GAL4/FRT stocks that allow for directed mosaics of mutations on 2L, 2R, 3L and 3R (Table 2). Each line carries an UAS-FLP, a follicular GAL4 driver and a FRT insertion. With a single cross of the appropriate FLP/GAL4/FRT line to a FRT-bearing mutant line, follicular mosaics for that mutant can be generated in the F_1. This provides a means to screen ~95% of the genome for roles in follicular development via directed mosaics.

Mosaic analysis of RTK signaling molecules supports follicular roles in axis patterning

Establishment of both A/P and D/V polarity within the oocyte depends upon signal transduction through Torpedo, the *Drosophila epidermal growth factor receptor* (Egfr) (Schupbach, 1987; Gonzalez-Reyes et al., 1995; Roth et al., 1995). Evidence exists supporting roles for RTK downstream molecules within the follicular epithelium for the establishment of axis polarity. Polarity disruptions, evident when the activity of these molecules is reduced within both the germline and the follicular epithelium, are not observed when the germline is lacking their activity; thereby supporting the notion that they are functioning within the follicular epithelium. Consistent with this, our screen uncovered previously unidentified alleles of *Drosophila Map kinase kinase* (*DMEK*) and the phosphatase Corkscrew (Csw). For three of the lethals that we tested, directed mosaic females laid eggs with ventralized chorions and had egg chambers with disruptions in A/P polarity. Complementation analysis with two of the lethal lines, 817 and 667, indicated that they were alleles of *csw*, while genetic mapping, complementation and transposon-mediated rescue analysis of the third lethal, 756, indicated that it was an allele of *DMEK*. DMEK has been previously implicated in D/V patterning through the use of a temperature-sensitive mutation (Hsu and Perrimon, 1994), while the role of Csw in establishing polarity has been demonstrated through hs-regulated Csw expression (Perkins et al., 1996). By identifying alleles of these loci through a directed mosaic screen, we have confirmed a tissue-specific and spatial requirement for these molecules in patterning both the A/P and D/V axes, consistent with previous hypotheses. Correlation of the location of MEK clones with the presence of a disrupted A/P axis also provided evidence to support the notion that the A/P axis organizing activity is generated by the posterior follicle cells (Fig 5).

A similar case for a somatic role of a negative regulator of RTK signaling, GAP1, has also been made for D/V patterning (Chou et al., 1993). During mid-oogenesis ventral follicular fates are specified by inactivation of the Ras-Raf-MEK

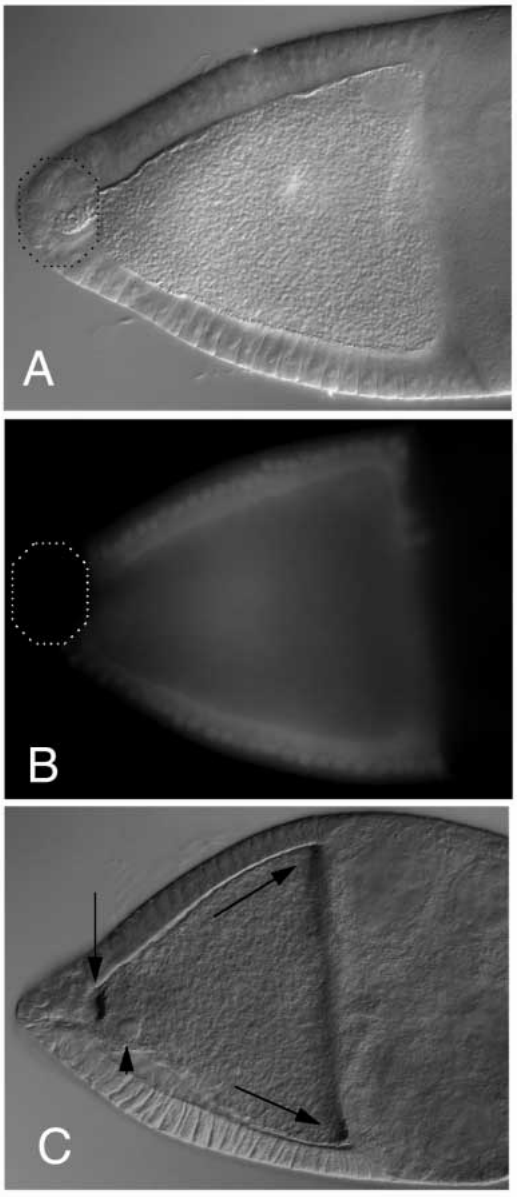


Fig. 5. Follicle cell clones of *MEK* result in disruptions in follicle cell fate and A/P polarity. (A,B) The same stage 10A egg chamber with a posterior follicle cell clone of *MEK* (marked with a dotted circle). (A) In the Nomarski image, a mispecification of posterior follicular fates is suggested by the rounding up of some posterior cells, while others are uncharacteristically invading the oocyte and pinching off a portion of the yolk-filled oocyte cytoplasm. (B) In the fluorescence image, the posterior location of the *MEK* follicle cell clone is marked by the absence of GFP expression. (C) A *bicoid in situ* hybridization to a similar stage 10 *MEK* clonal egg chamber. Normal localization of the *bicoid* message (arrows) is seen at the anterior, while a duplication of the anterior axis is evident by the ectopic localization of the *bicoid* message at the posterior. Arrowhead marks oocyte nucleus.

Table 2. FLP/FRT stocks for generating follicular clones

Chromosome	Genotype	FRT insertion site
X	(1) *y,w, FRT^{101}; e22c-GAL4, UAS-FLP/CyO*	14A-B
	(2) *y,w, FRT$^{9-2}$; e22c-GAL4, UAS-FLP/CyO*	18E
2L	(3) *w, FRT^{40A}; T155-GAL4, UAS-FLP*	40A
2R	(4) *w, FRT^{G13}; T155-GAL4, UAS-FLP*	42B
3L	(5) *w; e22c-GAL4, UAS-FLP/CyO; FRT^{2A}/TM3, Sb*	79D-F
3R	(6) *w; e22c-GAL4, UAS-FLP/CyO; FRT^{82B}*	82B

signaling cassette, proposed in part by the inhibitory action of GAP1. This in turn results in the production of a ventralizing activity during early embryogenesis. It is this activity that directs the graded nuclear to cytoplasmic transport of the Dorsal (Dl) protein, which regulates embryonic D/V axis formation (Morisato and Anderson, 1995). As a negative regulator of the Ras-Raf-MEK signaling pathway reduced GAP1 activity in the germline and follicular epithelium results in embryonic dorsalization. In contrast, germline reduction alone has no effect on D/V polarity, supporting its proposed role in follicular patterning (Chou et al., 1993). To extend these observations, we generated *e22c-GAL4*-directed mosaics of GAP1 in the follicular epithelium. From GAP1-directed mosaic females, we observed dorsalization of the embryonic cuticle. However, in addition to complete dorsalization, we also observed cases of extremely localized dorsalization (Fig. 6). Consistent with these observations, expression of the ventral molecular marker *twist* was lost in ventral regions in embryos derived from these females (data not shown).

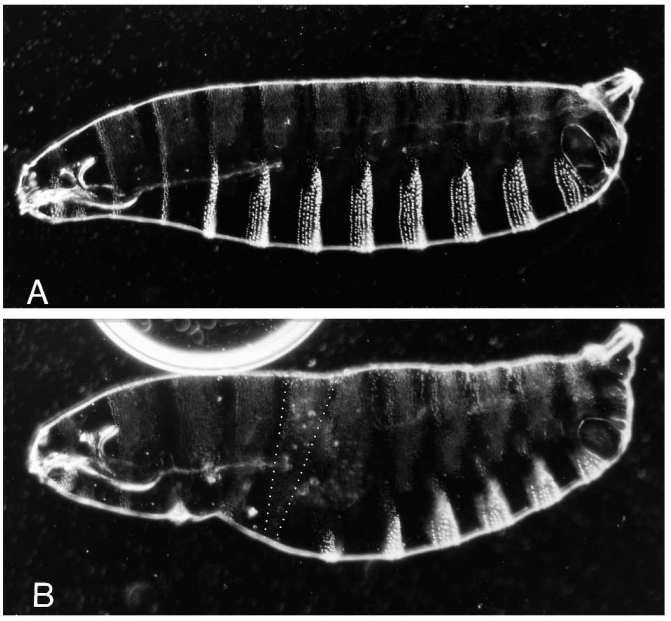


Fig. 6. Directed mosaics of *GAP1* result in localized embryonic dorsalization. (A,B) Dark-field micrographs of larval cuticles are shown. Anterior is to the left and dorsal up. (A) Wild-type embryo. The ventral surface is marked with thick highly reflective denticle bands, while the dorsal surface is marked with fine hairs. (B) The ventral region surrounding the second abdominal segment (marked with dotted lines) lacks a denticle band and, instead, the dorsal hairs extend all the way from the dorsal to the ventral surface.

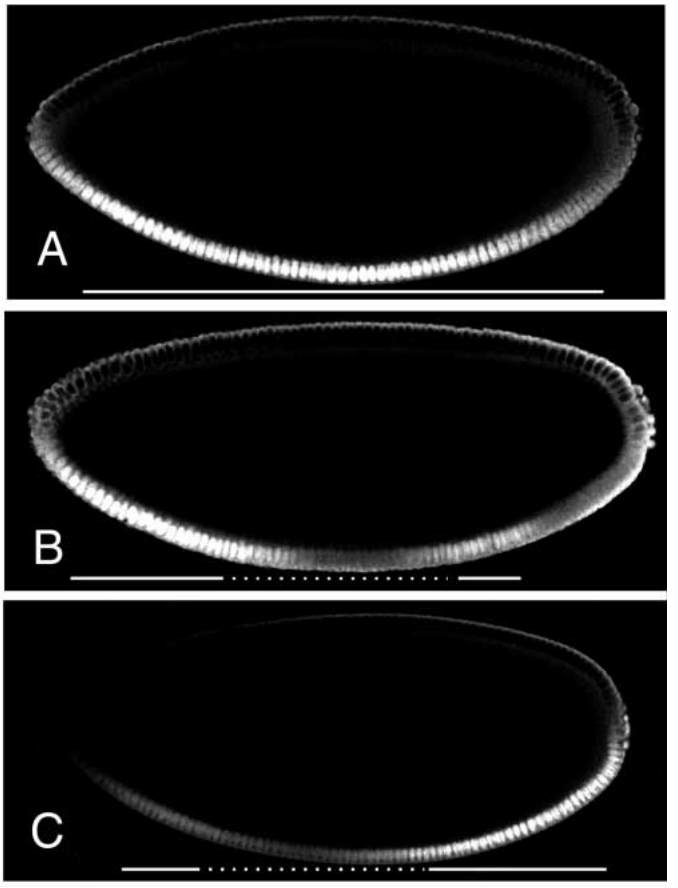


Fig. 7. Directed mosaics of *GAP1* result in localized disruptions of dorsal (Dl) nuclear localization. (A-C) Fluorescent immunohistochemistry of Dl nuclear/cytoplasmic localization in cellular blastoderm stage embryos oriented with anterior to the left and dorsal up. (A) Wild-type pattern of Dl localization. On the dorsal surface Dl is localized to the cytoplasm, evident as apical caps in the dorsal cells. In contrast, on the ventral surface, Dl is localized to the nucleus. (B,C) Embryos derived from directed mosaics of GAP1 with disruptions in Dl nuclear localization on the ventral surface. These disruptions are indicated by the hatched white lines. The transition zones between these ventral disruptions and the normal ventral nuclear localization do not show a sharp boundary, but instead show a graded translocation of Dl into its ventral nuclear localization pattern.

These results suggested that a localized loss of GAP1 activity within the follicular epithelium led to a localized inactivation of the embryonic ventralizing activity. Previous reports using embryos lacking the Toll receptor have suggested that this ventralizing activity is generated as a diffusible gradient from a ventral source (Stein et al., 1991; Stein and Nusslein-Volhard, 1992). Alternatively, recent results support the possibility in which the embryonic gradient of Dl localization could be generated by a ventral gradient within the follicular epithelium that transmits information in a cell-autonomous fashion (Queenan and Schupbach, 1997). This information would only be capable of diffusion in the absence of a receptor, but not in the presence of a wild-type receptor. By generating localized GAP1 clones (localized dorsalization) within the follicle cells and observing the embryonic nuclear translocation pattern of Dl, we were able to distinguish between these potential D/V patterning

mechanisms. In embryos derived from directed mosaic GAP1 mutant females, localized disruptions in the nuclear translocation of Dl were observed (Fig. 7B,C). Specifically, ventral regions without detectable nuclear Dl localization were observed. However, in these regions, sharp boundaries of Dl translocation were not present as is predicted from the cell-autonomous model. Rather, these disruptions were always accompanied by transition zones showing a graded translocation of Dl into the wild-type pattern, indicating a ventralizing activity capable of diffusion even in the presence of a wild-type Toll receptor (Fig. 7B,C).

DISCUSSION

Establishment of a directed mosaic system

As exemplified by the seminal screens for lethal mutations affecting embryonic pattern in *Drosophila* (Nusslein-Volhard and Wieschaus, 1980), the ability to genetically dissect the process of development has provided enormous insight into its underlying molecular mechanisms. It is this embryonic requirement for many of the molecules and signaling pathways that also contribute to later developmental stages that has hampered a similar systematic analysis of post-embryonic development. For such an analysis, one must be able to efficiently bypass the early/vital requirements of any locus to determine its function at later stages of development. By targeting the activity of the FLP recombinase in a spatial and temporal fashion, we have demonstrated that it is possible to efficiently generate directed mosaics for vital loci in most adult tissues.

Although the primary goal of this work was to establish a more directed approach for producing mosaics than previously available, our general adult screen compares favorably with an untargeted mosaic screen using FLP driven by a heat-shock promoter (Xu and Rubin, 1993). While that screen used X-irradiation to generate mutations and we used EMS, the frequency of lethal mutations was the same as for the screen reported here, one lethal mutation per chromosome arm. In contrast, in the Xu and Rubin screen, ~1% of the adult flies had visible defects – with ~0.6% having eye defects, while in our directed screen ~12% of the adults had visible defects, and 6.8% had eye defects. Thus, the directed mosaic technique yielded detectable phenotypes with ~12-fold higher frequency than the heat-shock generation method. While the rate of eye defects is sufficiently high by either method to feasibly conduct a screen, rarer classes of phenotypes, such as homeotic transformations and pattern duplications, were only detected with the directed mosaic method. Furthermore, another advantage in using the directed mosaic method for screening was the consistency with which mutant clones were produced. In contrast to heat-shock induction with which variation is seen both within an individual and a population, the use of GAL4-mediated FLP expression results in consistent and reproducible clones both within individuals and populations. For example, in flies with eye phenotypes, both eyes routinely displayed defects in our screens. Such consistency makes it easier to distinguish true genetic lesions from non-heritable developmental abnormalities, as well as to carry out phenotypic analyses.

Directed mosaic analyses of follicular patterning

Our two assays using viable markers gave consistent results regarding the production of mosaic tissue within the follicular epithelium. Temporal analysis of clonal production via *e22c-GAL4* yielded a number of relevant observations. First, shortly after eclosion most (>90%) egg chambers are mosaic containing small clones. Second, as the females age the frequencies of monoclonal egg chambers and ovarioles increase to reach maximal monoclonal frequencies of ~30%. These results indicate that temporally the system can be considered efficient from two distinct standpoints: the induction of small clones or monoclonal epithelial tissue. In addition, this temporal aspect provides us with a mechanism to control clonal size simply by regulating the age of females examined.

Previous work from a number of laboratories has provided an emerging model of how axis formation is established during oogenesis (for review see (Morisato and Anderson, 1996; Ray and Schupbach, 1996). Prior to stage 7 of oogenesis, a germline-derived signal, encoded by the TGF-alpha-like molecule, Gurken, specifies posterior follicle cell fates through the activity of the Top/DER RTK. This appears to occur through the activation of the downstream Ras-Raf-MEK signaling cassette within the posterior follicle cells. In response to this Grk-mediated signal, a secondary signal is sent from the posterior follicle cells back to the oocyte. The reception of this secondary signal then results in a reorganization of the microtubule network of the oocyte and the definition of the A/P axis. Concomitant with these events is a relocalization of the oocyte nucleus with its associated-Grk signaling activity to the dorsal-anterior cortex. In this new position, Grk and Top/DER-mediated signaling specifies dorsal follicle cell fates. The absence of this signal on the ventral surface results in the assumption of ventral follicular fates. From these follicle cells, a ventralizing activity is generated that is responsible for the activation of the Toll receptor. The activation of Toll then creates a ventral/dorsal gradient of nuclear to cytoplasmic Dorsal within the developing embryo to specify fates along the D/V axis. While a firm basis exists for this model of the serial relay of positional information, numerous points remain to be confirmed and questions answered with respect to the creation and relay of these signaling asymmetries.

Towards this end, we have used the directed mosaic system to provide further insight into how the follicular epithelium contributes to pattern formation. Specifically, we have directly supported the requirement for two members of the DER RTK signaling pathway, MEK and Csw, within the posterior follicular epithelium for axis organization. Furthermore, by limiting clones to the posterior of the follicular epithelium, we have established that it is posterior somatic cells that require RTK signaling activity for an A/P axis-organizing activity to be sent back to the germline-derived oocyte.

In addition, our mosaic analysis of GAP1 has provided the following observations. First, in embryos derived from GAP1-directed mosaic females a loss of Dl nuclear localization can be observed on the ventral surface. In addition, these ventral disruptions in Dl localization were always bordered by transition zones showing a graded translocation of Dl into the wild-type pattern. If the ventralizing signal were not diffusible then any disruptions in Dl nuclear localization would be bordered by sharp boundaries. In contrast, if the ventralizing signal was freely diffusible one would not expect to observe the loss of Dl nuclear localization in these embryos. In such cases, active signal would diffuse into regions unable to generate their own signal. Rather than support either of these extreme alternatives, our observations suggest a more moderate view: that active ventralizing signal is

capable of short-range diffusion even in the presence of the Toll receptor and that it is present in limiting amounts.

Directed mosaic screen for mutants affecting oogenesis

Finally, we have identified vital loci affecting a number of processes involving the development and organization of the follicular epithelium. As some of these loci have chorion phenotypes that represent D/V or A/P alterations, but have no effects on embryonic axis formation they are excellent candidates for downstream targets responsible for elucidating phenotypic subsets of the axis-forming signals. With directed mosaic stocks that allow for the identification of vital loci throughout ~95% of the genome, a genetic approach to follicular development and its contributions to oocyte/embryonic patterning is feasible. Moreover, as the specificity of clonal production relies upon a GAL4 driver, this approach takes advantage of an extensive collection of GAL4 lines throughout the *Drosophila* community. Thus, we anticipate this system providing an efficient, genetic means to study most developmental processes.

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REFERENCES

Ashburner, M. (1989). *Drosophila, A Laboratory Handbook*. New York: Cold Spring Harbor Press.

Brand, A. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brand, A. H. and Perrimon, N. (1994). Raf acts downstream of the EGF receptor to determine dorsoventral polarity during *Drosophila* oogenesis. *Genes Dev.* **8**, 629-639.

Brower, D. L. and Jaffe, S. M. (1989). Requirement for integrins during *Drosophila* wing development. *Nature* **342**, 285-287.

Calleja, M., Moreno, E., Pelaz, S. and Morata, G. (1996). Visualization of gene expression in living adult *Drosophila*. *Science* **274**, 252-255.

Chou, T.-B., Noll, E. and Perrimon, N. (1993). Autosomal *P[ovo^{D1}]* dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development* **119**, 1359-1369.

Chou, T. B. and Perrimon, N. (1992). Use of a yeast site-specific recombinase to produce female germline chimeras in *Drosophila*. *Genetics* **131**, 643-653.

Davis, I., Girdham, C. H. and O'Farrell, P. H. (1995). A nuclear GFP that marks nuclei in living *Drosophila* embryos: maternal supply overcomes a delay in the appearance of zygotic fluorescence. *Dev. Biol.* **170**, 726-729.

Duffy, J. B., Wells, J. and Gergen, J. P. (1996). Dosage-sensitive maternal modifiers of the *Drosophila* segmentation gene *runt*. *Genetics* **142**, 839-852.

Golic, K. and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* **59**, 499-509.

Golic, K. G. (1991). Site-specific recombination between homologous chromosomes in *Drosophila*. *Science* **252**, 958-961.

Gonzalez-Reyes, A., Elliot, H. and St Johnston, D. (1995). Polarization of both major body axes in *Drosophila* by gurken-torpedo signalling. *Nature* **375**, 654-658.

Harrison, D. and Perrimon, N. (1993). Simple and efficient generation of marked clones in *Drosophila*. *Current Biology* **3**, 424-433.

Hsu, J. C. and Perrimon, N. (1994). A temperature-sensitive MEK mutation demonstrates the conservation of the signaling pathways activated by receptor tyrosine kinases. *Genes Dev.* **8**, 2176-2187.

Kania, M. A., Bonner, A. S., Duffy, J. B. and Gergen, J. P. (1990). The *Drosophila* segmentation gene *runt* encodes a novel nuclear regulatory protein that is also expressed in the developing nervous system. *Genes Dev.* **4**, 1701-1713.

Margolis, J. and Spradling, A. (1995). Identification and behavior of epithelial stem cells in the *Drosophila* ovary. *Development* **121**, 3797-3807.

Martin, J. R., Raibaud, A. and Ollo, R. (1993). Torso-like: the putative ligand to torso receptor tyrosine kinase induces terminal pattern elements in *Drosophila* embryo. *Nature* **367**, 741-745.

Morisato, D. and Anderson, K.V. (1995). Signaling pathways that establish the dorsal-ventral pattern of the *Drosophila* embryo. In *Ann. Rev. Genetics* (ed. A. Campell, W. Anderson, and E. Jones), pp. 371-400. Palo Alto: Annual Reviews, Inc.

Nusslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795-801.

Perkins, L. A., Johnson, M. R., Melnick, M. B. and Perrimon, N. (1996). The non-receptor protein tyrosine phosphatase Corkscrew functions in multiple receptor tyrosine kinase pathways in *Drosophila*. *Dev. Biol.* (In press).

Perrimon, N. and Gans, M. (1983). Clonal analysis of the tissue specificity of recessive female sterile mutations of *Drosophila melanogaster* using a dominant female sterile mutation *Fs(1)K1237*. *Dev. Biol.* **100**, 365-373.

Qeenan, A. M. and Schupbach, T. (1997). Ectopic activation of *torpedo/EGFr*, a *Drosophila* receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. *Development* **124**, 3871-3880.

Ray, R.P. and Schupbach, T. (1996). Intercellular signaling and the polarization of body axes during *Drosophila* oogenesis. *Genes Dev.* **10**, 1711-1723.

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

Roth, S., Neuman-Silberberg, F. S., Barcelo, G. and Schupbach, T. (1995). cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. *Cell* **81**, 967-978.

Rørth, P. (1996). A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93**, 12418-12422.

Sanson, B., White, P. and Vincent, J.-P. (1996). Uncoupling cadherin-based adhesion from *wingless* signalling in *Drosophila*. *Nature* **383**, 627-630.

Savant-Bhonsale, S. and Montell, D. J. (1993). *torso-like* encodes the localized determinant of *Drosophila* terminal pattern formation. *Genes Dev.* **7**, 2548-2555.

Schupbach, T. (1987). Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of the egg shell and embryo in *Drosophila melanogaster*. *Cell* **49**, 699-707.

Siegfried, E., Chou, T. B. and Perrimon, N. (1992). wingless signaling acts through zeste-white 3, the *Drosophila* homolog of glycogen synthase kinase-3, to regulate engrailed and establish cell fate. *Cell* **71**, 1167-1179.

Simpson, P., el Messal, M., Moscodo del Prado, J. and Ripoll, P. (1988). Stripes of positional homologies across the wing blade of *Drosophila melanogaster*. *Development* **103**, 391-401.

Spradling, A. (1986). P element-mediated transformation. In *Drosophila, A Practical Approach*, vol. (ed. D. B. Roberts), pp. 175-198. New York: IRL Press.

Stein, D. and Nusslein-Volhard, C. (1992). Multiple extracellular activities in *Drosophila* egg perivitelline fluid are required for establishment of embryonic dorsal-ventral polarity. *Cell* **68**, 429-440.

Stein, D., Roth, S., Vogelsang, E. and Nusselin-Volhard, C. (1991). An activity present in the perivitelline space of the *Drosophila* egg determines the dorsoventral axis of the embryo. *Cell* **65**, 725-735.

Stevens, L. M., Frohnhofer, H. G., Klinger, M. and Nusslein-Volhard, C. (1990). Localized requirement for *torso-like* expression in follicle cells for development of terminal anlagen of the *Drosophila* embryo. *Nature* **346**, 660-663.

Struhl, G. and Basler, K. (1993). Organizing activity of wingless protein in *Drosophila*. *Cell* **72**, 527-540.

Xu, T. and Rubin, G. R. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Yeh, E., Gustafson, K. and Boulianne, G. L. (1995). Green fluorescent protein as a vital marker and reporter of gene expression in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **92**, 7036-7040.

Yoffe, K. B., Manoukian, A. S., Wilder, E. L., Brand, A. H. and Perrimon, N. (1996). Evidence for *engrailed*-independent *wingless* autoregulation in *Drosophila*. *Dev. Biol.* **170**, 636-650.