INTRODUCTION

Formation of the *Drosophila* egg involves the contribution of two different ovarian cell types, the nurse cell-oocyte complex of germ-line origin derived from the embryonic pole cells, and the follicle cells derived from the embryonic mesoderm (King, 1970). The early separation of these two cell types makes it possible to generate chimeras that have germ-line and follicle cells of different genotypes. Generation of such chimeras has been important for two sets of experiments. First, they allow the determination of the tissue specificity of recessive female-sterile (fs) mutations (Wieschaus et al., 1981; Perrimon and Gans, 1983). Saturation screens have provided us with a large collection of fs mutations involved during oogenesis and embryonic patterning (Gans et al., 1975; Mohler, 1977; Komitopoulou et al., 1983; Perrimon et al., 1986; Schupbach and Wieschaus, 1989). Due to the dual origin of the cell types that form the egg and their complex interactions in oogenesis, it is critical to determine the tissue specificity of these fs mutations by generating germ-line mosaics. Second, germ-line chimeras allow one to analyze the maternal effect of zygotic lethal mutations (Perrimon et al., 1984, 1989). A critical aspect of both techniques is the ability to distinguish the eggs derived from homozygous mutant cells from the otherwise wild-type eggs. We have cloned the ovoD1 mutation into a P-element vector and recovered fully expressed P[ovoD1] insertions on each autosomal arm. We describe the generation of these P[ovoD1] strains as well as demonstrate their use in generating germ-line chimeras. Specifically, we show that the Gap1 gene, which encodes a *Drosophila* homologue of mammalian GTPase-activating protein, is required in somatic follicle cells for embryonic dorsoventral polarity determination.

Key words: *Drosophila*, oogenesis, mosaic, female-sterile

SUMMARY

The ‘dominant female-sterile’ technique used to generate germ-line mosaics in *Drosophila* is a powerful tool to determine the tissue specificity (germ line versus somatic) of recessive female-sterile mutations as well as to analyze the maternal effect of recessive zygotic lethal mutations. This technique requires the availability of germ-line-dependent, dominant female-sterile (DFS) mutations that block egg laying but do not affect viability. To date only one X-linked mutation, ovoD1 has been isolated that completely fulfills these criteria. Thus the ‘DFS technique’ has been largely limited to the X-chromosome. To extend this technique to the autosomes, we use the ‘dominant female-sterile’ technique used to generate germ-line mosaics in *Drosophila* is a powerful tool to determine the tissue specificity (germ line versus somatic) of recessive female-sterile mutations as well as to analyze the maternal effect of recessive zygotic lethal mutations. This technique requires the availability of germ-line-dependent, dominant female-sterile (DFS) mutations that block egg laying but do not affect viability. To date only one X-linked mutation, ovoD1 has been isolated that completely fulfills these criteria. Thus the ‘DFS technique’ has been largely limited to the X-chromosome. To extend this technique to the autosomes, we have cloned the ovoD1 mutation into a P-element vector and recovered fully expressed P[ovoD1] insertions on each autosomal arm. We describe the generation of these P[ovoD1] strains as well as demonstrate their use in generating germ-line chimeras. Specifically, we show that the Gap1 gene, which encodes a *Drosophila* homologue of mammalian GTPase-activating protein, is required in somatic follicle cells for embryonic dorsoventral polarity determination.

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INTRODUCTION

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Two techniques are currently used to generate germ-line chimeras: pole cell transplantation in which mutant pole cells are transferred into an otherwise wild-type donor (Illmensee, 1973) and mitotic recombination (Wieschaus and Szabad, 1979). A critical aspect of both techniques is the ability to distinguish the eggs derived from homozygous mutant cells from the otherwise wild-type eggs. In the case of pole cell transplantation, this can be easily done by injecting the mutant pole cells into an animal that does not develop germ cells. Females lacking germ cells can be generated by using recessive or dominant female-sterile mutations that prevent pole cell formation or affect germ cell development. In the case of mitotic recombination, the most successful approach has been the use of germ-line-dependent DFS that do not lay eggs (the ‘DFS’ technique; Wieschaus, 1980; Perrimon and Gans, 1983). A mitotic recombination event occurring in the germ cells of females heterozygous for DFS will result in recombinant germ-line daughter cells that have eliminated the DFS mutation and thus can develop into normal eggs. If the female is trans-heterozygous for both a specific mutation (fs or zygotic lethal) and the DFS mutation, the mitotic exchange will result in simultaneous loss of the DFS mutation and homozygosity of the mutation. The tissue specificity of a fs mutation or the maternal effect phenotype of a zygotic lethal mutation can then be analyzed by examining the phenotypes of eggs and embryos derived from these germ-line clones.

The most widely used DFS mutation is the X-linked ovoD1 mutation (or Fs(1)K1237, Busson et al., 1983; Perrimon, 1984). Females heterozygous for ovoD1 never lay
eggs because they have rudimentary ovaries in which oogenesis is blocked at an early stage. The female sterility associated with the \textit{ovo}^{D1} mutation is strictly germ-line dependent such that flies carrying this mutation are perfectly viable and wild-type germ cells in an \textit{ovo}^{D1} heterozygous female produce wild-type eggs. In addition, germ cells heterozygous for \textit{ovo}^{D1} develop poorly such that, when a germ cell has eliminated \textit{ovo}^{D1}, it has a growth advantage and the resulting clone can usually give rise to a fully developed ovary (Busson et al., 1983; Perrimon, 1984). This overproliferation of wild-type cells in an \textit{ovo}^{D1} background has been a major advantage in using \textit{ovo}^{D1} to generate germ-line mosaics.

The frequency at which germ-line clones are recovered is an important consideration in designing experiments. Mitotic recombination events induced in \textit{ovo}^{D1} heterozygous animals following X-ray irradiation are generated at a frequency of approximately 5% of mosaic females among heterozygous \textit{ovo}^{D1} females (Perrimon, 1984). Recently, we have combined the use of the site-specific recombination system (Golic and Lindquist, 1989; Golic, 1991) to develop the 'FLP-DFS' technique (Chou and Perrimon, 1992) where the frequency of mosaics reaches almost 100%.

Screens for autosomal mutations associated with female sterility have identified a few DFS mutations, some of which have been used occasionally to generate germ-line mosaics (Yarger and King, 1971; Erdelyi and Szabad, 1989; Szabad et al., 1989). These DFS mutations in some cases affect viability or lay some eggs making their use difficult in detecting germ-line chimeras. In addition, clones induced using DFS mutations such as \textit{Fs(2)D} (Yarger and King, 1971) and \textit{Fs(2)I} (Szabad et al., 1987, 1989) are small because none show the regulation phenomena observed in the background of the \textit{ovo}^{D1} mutation (N. P. unpublished data).

To overcome the lack of appropriate autosomal DFS mutations to generate germ-line chimeras, we first attempted to create DFS mutations by selectively expressing the A chain of diphtheria toxin (DT-A) in the female germ line. DT-A has been shown to induce cell death in a cell autonomous manner (Palmiter et al., 1987) such that dominant female sterility could be achieved by expressing DT-A selectively in female germ cells. We tested a number of female germ-line-specific promoter elements including part of the promoter of the \textit{Drosophila} hsp26 gene (Frank et al., 1992) and an element from the \textit{ovarian-tumor} promoter (Comer et al., 1992) to drive the expression of DT-A in the female germ line. In addition, we used the germ-line-specific splicing intron of the \textit{P}-element transposase (Laski and Rubin 1989) or the sex-specific splicing intron of the \textit{transfor}mer gene (Sosnowski et al., 1989) to further restrict female germ-line cell expression of the DT-A gene. However, we failed to recover \textit{P}-element transformants of these constructs due to the strong toxicity of DT-A resulting from transient expression in injected embryos. After laborious attempts to obtain transformants of these constructs, we decided that transposition of the \textit{ovo}^{D1} mutation may be a more straightforward approach. Since early attempts to translocate genetically the \textit{ovo}^{D1} mutation failed due to the presence of regions of unusual genetic properties around the \textit{ovo} gene (Oliver et al., 1988), we decided that transposition of a cloned \textit{ovo}^{D1} mutation following \textit{P}-element transformation might constitute a better approach. These experiments were possible since the \textit{ovo} gene has been cloned (Mevel-Ninio et al., 1991; Garfinkel et al., 1992). In this paper, we report the successful recovery of autosomal \textit{P}-element insertions that carry the \textit{ovo}^{D1} mutation and demonstrate their use in generating germ-line mosaics.

**MATERIAL AND METHODS**

**Construction and isolation of an \textit{ovo}^{D1} cosmid**

\textit{ovo}^{D1} genomic DNA was isolated from \textit{ovo}^{D1} \texttt{Yv24/Y} males and prepared by standard methods (Sambrook et al., 1989). The construction of the \textit{ovo}^{D1} cosmid library followed the protocol of Ish-Horowicz and Burke (1981). \textit{ovo}^{D1} genomic DNA was partially digested with \textit{SaNu}3A and ligated into the pCosPeR vector (V. Pirrotta, personal communication) digested with \textit{Bam}HI. In vitro packaging was performed using Gigapack II XL packaging extract from Stratagene. After titration of the cosmid library, the primary colonies were screened without amplification using 4 different PCR probes which cover the entire \textit{ovo} transcription unit (Fig. 1). Coordinates for these primers followed that described in Mevel-Ninio et al. (1991). The 4 pairs of oligomers are as follows. \texttt{o/1:} 5'-ATCGACTCTCCTATACGTTG-3' and \textit{ovo}-740 primer: 5'-CAATACAAAGGATGAAA-3' produce a 740bp fragment; \texttt{o/2:} 5'-ATCGACTCTCCTATACGTTG-3' and \texttt{o/2:} 5'-GATGCTTCAAGAAGGTTT-3' produce a 654bp fragment; \texttt{o/3:} 5'-GATGCTTCAAGAAGGTTT-3' and \texttt{o/4:} 5'-GATGCTTCAAGAAGGTTT-3' produce a 654bp fragment; \texttt{o/3:} 5'-GATGCTTCAAGAAGGTTT-3' and \texttt{o/4:} 5'-GATGCTTCAAGAAGGTTT-3' produce a 638bp fragment. The 638bp PCR fragment was used first as a probe for primary colony screening. Direct colony PCR screening (Hamilton et al., 1991) was performed thereafter. Colonies surrounding the positive signals were scooped into 100 µl LB solution. Using the primers listed above, PCR reactions were performed immediately in 25 µl solution containing 1 µl colony suspension. Primers were used at 250 nM. Amplifications were done first with 2 minutes 30 seconds at 94°C for bacterial cell lysis followed by 30 cycles at 94°C for 1 minute; 54°C for 1 minute; 72°C for 3 minutes. Colonies that were positive with all 4 PCR products were used for the secondary screen using both 935bp and 654bp fragments separately as probes. One positive colony from this screen was confirmed again by PCR and used for the preparation of cosmID DNA.

**Subcloning of \textit{ovo}^{D1} genomic DNA fragment**

A 7.2 kb genomic DNA fragment containing the putative 5 kb \textit{ovo}^{+} mRNA transcription unit can rescue the female sterility phenotype of \textit{ovo} mutations (Mevel-Ninio et al., 1991). A 10.5 kb \textit{ovo}^{D1} genomic DNA fragment containing the \textit{ovo}^{D1} counterpart of the 7.2 kb rescuing fragment and approximately 3 kb downstream was first cloned into the pCaSpeR2 vector (Thummel and Pirrotta, 1988). The \textit{ovo}^{D1} cosmid DNA was partially digested with \textit{Bgl}II followed by complete digestion with \textit{Eco}RI to generate the expected 10.5 kb fragment. The ligation reaction was done in Nuseive-agarse gel (Sheen, 1993) containing both the 10.5 kb \textit{Bgl}II-\textit{Eco}RI insert and the pCaSpeR2 vector digested with \textit{Bgl}II and \textit{Eco}RI. This \textit{ovo}^{D1} subclone, pD1B2R, was partially digested with \textit{Not}I and end-filled with the Klenow fragment of DNA polymerase. The resulting clone, pD1B2R#225, has no \textit{Not}I site in the pCaSpeR2 vector sequences. A 12 kb \textit{Not}I-\textit{Bgl}II DNA fragment isolated from \textit{Bgl}II partial and \textit{Not}I complete digestion of \textit{ovo}^{D1} cosmID DNA, Cos-P[\textit{ovo}^{D1}]	extsuperscript{S1}, was cloned into \textit{Not}I and \textit{Bgl}II.
digested pD1B2R#225 vector. The resulting 18 kb ovoD1 clone, pD1B2NR, contains 8kb upstream of the 7.2 kb Sal-HindIII region and approximately 3kb downstream. Another 7.2 kb subclone, pD1B2H, containing only the 7.2 kb ovoD1 Sal-HindIII counterpart was cloned. The ovoD1 cosmid DNA was then partially digested with HindIII and completely digested with BglII. The purified 7.2 kb fragment was introduced into pCaSpeR2 vector partially digested with HindIII and completely digested with BglII. Basic molecular biology manipulations were done as described in Sambrook et al. (1989).

**P-element transformation**

DNA constructs used for microinjection were purified through an EtBr-CsCl gradient. The EtBr-DNA band was extracted four times with 1:1 isopropanol-Tris-EDTA solution saturated with NaCl to remove EtBr. DNA was then precipitated with EtOH and resuspended in TE. Further extraction was performed at least three times with phenol/chloroform. The purified DNA was then re-precipitated with EtOH and used for microinjection. Modified microinjection was done as described by Chou et al. (1987). The host strains used for injection were y w or y y; D2-3, Sb/TM6 (Robertson et al., 1988). Both transposase helper plasmids, ptt25.7w (Karess and Rubin, 1984) and pa2-3 (Laski et al., 1986), were used in different injection experiments. Transformants were identified by rescue of the white eye color.

**Transposase stocks**

The ‘jumpstarter’ strain used is D2-3, P[ry+]; D2-3, which carries a defective P-element on the third chromosome at 99B which constitutively expresses high levels of transposase but can not itself transpose (Robertson et al., 1988). The stocks, ry506, D2-3 and CyO:S; ry506, D2-3, Sb/TM6, that provide a source of transposase activity were obtained from the Bowling Green Stock Center. The stock carrying D2-3 on the TM3 balancer chromosome (TM3, Sb ry D2-3/Dr(3R)C7, ry506) was obtained from G. Reuter and J. Szabad.

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

**Autosomal P[ovoD1] stocks**

Autosomal P[ovoD1] dominant female mutations are kept as stocks using dominant male-sterile mutations. Second chromosome P[ovoD1] insertions are kept as wY; P[ovoD1]/CyO males crossed with Sp Mst(2)M bwP/CyO females. Third chromosome P[ovoD1] insertions are kept as wY; P[ovoD1]/TM3, Sb males crossed with ru h st B2D ss e/TM3, Sb females. The dominant male-sterile stocks Mst(2)M and B2D were obtained from D. Lindsley and M. Fuller, respectively (D. Lindsley, personal communication; Kempheus et al., 1980).

Concurrently, P[ovoD1] dominant female insertions are also kept as wY; P[ovoD1]/CyO and wY; P[ovoD1]/Sco males crossed with w/w; CyO/Sco females (for the second chromosome), and wY; P[ovoD1]/TM3, Sb and wY; P[ovoD1]/Cxd males crossed with w/w; TM3, Sb/Cxd females (for the third chromosome).

**In situ hybridization**

In situ hybridization to polytene chromosomes was performed as described in Chou and Perrimon (1992). The probe used is the pP[Δw5+1] plasmid containing FRT sequences, the mini-w (Δw5) gene and the P-element terminal inverted repeat sequences (Golic and Lindquist, 1989).

**Egg-laying ability test**

10-20 females, heterozygous for a P[ovoD1] insertion, were mated to five males in a vial for at least 6 days and examined for their egg-laying ability. When eggs were found in a vial, the number of females laying eggs was determined by looking for the presence of mature egg chambers either by visual examination of the abdomen or following ovary dissection.

**Induction of germ-line clones**

To induce germ-line clones, first instar larvae (24 to 48 hours old) from the appropriate cross were irradiated at a constant dose of 1000 rads (Torrex 120D X-ray machine; 100 kV, 5 mA, 3 mm aluminum filter) as described in Perrimon et al. (1984).

**Examination of embryonic phenotypes**

Females that carry germ-line clones were detected by either examining their egg-laying ability or by the presence of developed ovaries. Eggs were scored as unfertilized if no embryonic development was detectable following dechorionation in 50% bleach. Cuticles were prepared in Hoyers’ mountant as previously described (van der Meer, 1977). Embryos were examined by both phase-contrast and dark-field microscopy.

**Mutations used for germ-line clonal analysis**

rhd5D5/TM6B and rhd5AS/TM6B (Freeman et al., 1992), y w; rhd543 e/TM6B, Tb (Mayer and Nusslein-Volhard, 1988), sev5B; Sox1122/SMa4 (Rogge et al., 1991), st e Tp444/TM3 (Anderson et al., 1985), Gap18B1/TM6 and w; Gap182B3/TM3, Sb (Gaul et al., 1992), and w; mipw+/TM6C (Buckles et al., 1992) were used for germ-line clonal analyses.

**RESULTS**

**Recovery of P[ovoD1] transformants**

The ovo gene is located at cytological position 4E1-2. Genetic analysis of this region has shown that ovo may be part of a gene complex that includes the shaved-baby (svb) gene. Many ovoD1 revertants are also mutant at the svb locus, which maps less than 0.5 map unit from ovo, suggesting that both genes may share common regulatory or coding sequences (Olivier et al., 1988; Mevel-Ninio et al., 1991; Garfinkel et al., 1992). Since the nature of the ovoD1 mutation, as well as the regulatory regions of ovo, have not yet been defined, we decided to generate a P-element transposon that contains a fragment of ovoD1 genomic DNA as large as possible for germ-line transformation. Since recovery of transformants using a Cos-P vector that contains close to 40 kb of DNA has been previously described (Haenlin et al., 1985), we attempted to introduce a Cos-P[ovoD1] into the fly genome. A cosmid, Cos-P[ovoD1]S22-1, was isolated as the result of a direct PCR screen (Materials and Methods). This cosmid encompasses the ovoD1 counterpart of the ovo+ 7.2 kb fragment, which can rescue the female sterility phenotype of ovo mutation (Mevel-Ninio et al., 1991) along with approximately 20 kb of upstream and 10 kb of downstream DNA (Fig. 1). 6,000 embryos were injected with Cos-P[ovoD1]S22-1, but no transformants were recovered (Table 1).

Three subclones of the ovoD1 gene, pD1B2NR, pD1B2R and pD1B2H (Fig. 1), were subsequently derived from Cos-P[ovoD1]S22-1 and used for P-element-mediated transformation. All of these plasmids were associated with high toxicity as shown by the poor recovery of larvae and adults from the injected embryos (see Table 1). We were not able to recover transformants of pD1B2R and pD1B2H (Table 1); however, three independent G0 males derived from pD1B2NR injec-
Figure 1. Cloning of ovoD1. The position of the ovoD1 genomic fragments used in the P-element transformation experiments are shown with respect to the extent of the ovo 7.2 kb rescuing fragment. This fragment contains the proposed 5 kb ovo+ mRNA transcription unit and rescues the female sterility phenotype of ovo− mutations (Mevel-Ninio et al., 1991). The 7.2 kb ovo fragment and the position of the four PCR products used for screening the cosmid library are indicated. Only the restriction sites used in the plasmid subcloning (see Material and Methods) are shown. For a more detailed map see Mevel-Ninio et al. (1991) and Garfinkel et al. (1992). The zero point demarcates the SalI site as described in Mevel-Ninio et al. (1991). The locations of SalI, BglII, NotI, HindIII, EcoRI and PstI restriction sites in the Cos-P[ovoD1]S23-1 clone are similar to those reported previously (Mevel-Ninio et al., 1991; Garfinkel et al., 1992). The Cos-P[ovoD1]S23-1 DNA contains 20 kb of DNA upstream of and 18 kb of DNA downstream of the SalI site. The extent of the three subclones derived from Cos-P[ovoD1]S23-1 is shown.

Table 1. Recovery of P[ovoD1] transformants

<table>
<thead>
<tr>
<th>Construct (insert size)</th>
<th>Strain/helper</th>
<th>Number injected</th>
<th>Number recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5 kb)</td>
<td>y w/pA2-3</td>
<td>150</td>
<td>24</td>
</tr>
<tr>
<td>S23-1 cosmids (38 kb)</td>
<td>y w/pA2-3</td>
<td>4,971</td>
<td>385</td>
</tr>
<tr>
<td>pD1B2NR (18 kb)</td>
<td>Δ2-3</td>
<td>2,354</td>
<td>21</td>
</tr>
<tr>
<td>pD1B2R (10.5 kb)</td>
<td>Δ2-3</td>
<td>407</td>
<td>19</td>
</tr>
<tr>
<td>pD1B2H (7.2 kb)</td>
<td>Δ2-3</td>
<td>2,119</td>
<td>37</td>
</tr>
</tbody>
</table>

Microinjections were performed as described in Chou et al. (1987). In control experiments, using a 5 kb plasmid at a concentration of 200 μg/ml, 30% of the injected embryos (E) hatch and 50% of the larvae (L) give rise to fertile G0 adults. 20% of the G0 adults are usually transformed. We found that ovoD1 plasmid subclones are toxic to injected embryos especially when injected into the Δ2-3 strain (y w; Δ2-3, Sh/TM6) or using the pA2-3 helper injected into a y w strain (Laski et al., 1986). The toxicity is apparent from the low hatching rate (8 to 12%) and from the low eclosion rate (less than 10% of the hatched larvae). The toxicity effect was lower when the germ-line-specific helper, prt25.7w, was used (Karess and Rubin, 1984).

DNA concentrations for injection are: 400-500 μg/ml for both the S23-1 cosmids and pD1B2NR subclone, 100-200 μg/ml for both pD1B2R and pD1B2H subclones, and 100-200 μg/ml for the transposase helpers in the final injection solution.

*In this experiment, 30 μg/ml of pD1N2BR was injected.

The number of G0 adults includes both males and females. Lines were not established from the G0 females since the presence of the ovoD1 mutation in the germ line of G0 females is expected to prevent egg formation.

Most females (94%) that carry one copy of the P[ovoD1]2 transposon have atrophic ovaries, a phenotype similar to females heterozygous for ovoD1. However, the remaining 6% females lay some abnormal eggs which never hatch (Table 2). These eggs have fused dorsal appendages and are usually flaccid, a phenotype also associated with two dominant alleles at the ovo locus, ovoD2 and ovoD3 (Busson et al., 1983; Perrimon, 1984). ovoD2+ females lay very few eggs which are always flaccid, while ovoD3+ females lay a lot of eggs with fused filaments which usually become flaccid. Both ovoD2 and ovoD3 behave as antimorphic mutations. First, their female sterility can be suppressed by the presence of two copies of the ovo+ gene; and, second, both mutations in trans with a loss of function ovo− mutation have atrophic ovaries, a phenotype also found in homozygous ovo− females (Busson et al., 1983; Oliver et al., 1987). The nature of the ovoD1 mutation is less straightforward. ovoD1 females that carry up to three copies of the ovo+ gene are sterile; however, their atrophic ovaries are larger than those of ovoD1 females that carry 1 or 2 copies of the ovo+ gene. These dosage experiments suggest that ovoD1, like ovoD2 and ovoD3, is an antimorphic mutation (Busson et al., 1983).

Since females that carry the autosomal P[ovoD1]2 insertion carry two X-linked copies of the ovo+ gene, we reasoned that the incomplete sterility of P[ovoD1]2 may be due to a lower expressivity of the transposed P[ovoD1] gene. A reduced amount of toxic ovoD1 product may lead to a weaker DFS phenotype reminiscent of the sterility phenotypes associated with both the ovoD2 and ovoD3 mutations. To test this hypothesis, we generated flies that carry the autosomal P[ovoD1]2 insertion in the presence of a single copy of the ovo+ gene. This was achieved by using the loss-
of-function mutation, ovoS1, which behaves as a null ovo- mutation (Oliver et al., 1987). ovoS1/+; P[ovoD1] insertion females had atrophic ovaries and significantly reduced egg-laying ability (Table 3), indicating that +/+; P[ovoD1] insertion females laid eggs due to incomplete expressivity of the transposed ovoD1 mutation. This result is consistent with the proposed antimorphic nature of the ovoD1 mutation (Busson, et al., 1983; Perrimon, 1984).

**Table 2. Recovery of autosomal [ovoD1] insertions**

<table>
<thead>
<tr>
<th>Insertions</th>
<th>Chromosomal location</th>
<th>New [ovoD1] insertions</th>
<th>P[ovoD1] selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[ovoD1]2</td>
<td>3L; 70B-C</td>
<td>II 12 6</td>
<td>P[ovoD1]+(2L; 27E-28A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III &gt;80 8</td>
<td>P[ovoD1]+ (2R; 55D-E)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X 5 0</td>
<td>P[ovoD1]+ (3L; 70A-E)</td>
</tr>
<tr>
<td>P[ovoD1]+(S1)</td>
<td>X</td>
<td>II, III 0 0</td>
<td></td>
</tr>
<tr>
<td>P[ovoD1]+(P)</td>
<td>X</td>
<td>II, III 0 0</td>
<td></td>
</tr>
<tr>
<td>P[ovoD1]+(L)</td>
<td>2L; 27E-28A</td>
<td>II 21 1</td>
<td>P[ovoD1]+(2L; 28A and 30D)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III 1 0</td>
<td>P[ovoD1]+(3LX)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II(CyO) 1 0</td>
<td>P[ovoD1]+(2R; 55B-D)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P[ovoD1]+(2R)</td>
</tr>
<tr>
<td>P[ovoD1]+(CyO)</td>
<td>CyO</td>
<td>III 16 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III 6 3</td>
<td></td>
</tr>
<tr>
<td>P[ovoD1]+(Cl)</td>
<td>3R; 98A-B</td>
<td>III &gt;25 0</td>
<td>P[ovoD1]+(3R; 98A-B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P[ovoD1]+(3R; ND)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P[oboD1]+(3X)</td>
</tr>
</tbody>
</table>

The number of novel P[ovoD1] insertions (N), their chromosomal segregation (Chrm.) and the number mapped to polytene chromosomes (N hyb), generated during the course of the various transposition experiments (see Fig. 2) is indicated. See Fig. 2 and text for further details. ND, Not determined.

**Table 3. Egg-laying abilities associated with [ovoD1] autosomal insertions**

<table>
<thead>
<tr>
<th>Insertions</th>
<th>+/+; D1/+</th>
<th>+/+; D1/Bal</th>
<th>Total</th>
<th>S1/+; D1/+</th>
<th>S1/+; D1/Bal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P[ovoD1]+</td>
<td>0/667</td>
<td>0/225</td>
<td>0/892</td>
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<td></td>
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<tr>
<td></td>
<td>P[ovoD1]+</td>
<td>1/374</td>
<td>0/263</td>
<td>1/637</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2R</td>
<td>P[ovoD1]+</td>
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<td>8/186</td>
<td>20/385</td>
<td>0/320</td>
<td>1/393</td>
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<tr>
<td></td>
<td>P[ovoD1]+</td>
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<td>0/381</td>
<td>1/947</td>
<td></td>
<td></td>
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<tr>
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<td>P[ovoD1]+</td>
<td>0/458</td>
<td>0/345</td>
<td>0/803</td>
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</tr>
<tr>
<td>3L</td>
<td>P[ovoD1]+</td>
<td>6/103</td>
<td>6/103</td>
<td>1/130</td>
<td>1/112</td>
<td>2/422</td>
</tr>
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<td>0/1576</td>
<td>0/1607</td>
<td>0/362</td>
<td>0/362</td>
</tr>
<tr>
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<td>P[ovoD1]+</td>
<td>5/295*</td>
<td>0/325</td>
<td>5/1197</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P[oboD1]+</td>
<td>2/593</td>
<td>0/433</td>
<td>2/1026</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The egg-laying ability of females that carry P[ovoD1] insertions in various mutant backgrounds is indicated. Female sterility is represented by the number of females that lay eggs the total number of females tested. D1 represents the P[ovoD1] insertion used, S1 is the loss-of-function ovo allele and Bal represents the balancer chromosomes (CyO for the second and TM3, Sb for the third).

A +/+; D1/+ genotype corresponds to a female carrying a P[ovoD1] insertion in the presence of two wild-type copies of the ovo gene on the X-chromosome. Similarly, a S1/+; D1/+ genotype corresponds to a female carrying a P[ovoD1] insertion in the presence of a single wild-type copy of ovo. Females heterozygous for a P[ovoD1] insertion sometimes lay eggs due to the occurrence of spontaneous mitotic recombination events in their germ line. These events occur at the frequency of 0.1-1% (Wieschaus and Szabad, 1979; Perrimon and Gans, 1983) and can be prevented by the presence of an appropriate balancer chromosome (Bal). The egg-laying ability of some P[ovoD1] females that carry a fully expressed insertion can be accounted for by these events.

Flies that carry a P[ovoD1] insertion are perfectly viable and morphologically normal. Leaky P[ovoD1] females can have either one or two ovaries which can range from a few ovarioles with vitellogenic oocytes to a fully developed ovary. The morphology of the eggs laid by P[ovoD1] females can be wild-type, flaccid or with fused dorsal appendages. These eggs can in some cases produce normal larvae and adults.

*In this experiment we did not determine the genotype of the females that produced eggs.

**New P[ovoD1] insertions are associated with incomplete expressivity**

P-element transposons can be mobilized using the Δ2-3 transposase (Robertson et al., 1988). These transposition events can be followed using the w* marker carried by the transposon. Flies heterozygous for the P[ovoD1] insertion have pale-orange eyes. To recover additional P[ovoD1] autosomal insertions, we first mobilized the original
recovered among flies with darker eye colors, we selected insertions associated with darker eye colors that segregated with the \( P[ovo^{D1}13] \) or \( P[ovo^{D1}]12 \) chromosomes. From the above transposition of the \( P[ovo^{D1}]13 \) element, 21 lines were recovered with dark eye colors. Two of them, \( P[ово^{D1}13X0] \) and \( P[ovo^{D1}13X13] \), showed the fully expressed \( ovo^{D1} \) DFS phenotype. In situ hybridization detected two signals on the 3R chromosome, one at 28A, which most likely corresponds to the original site of \( P[ovo^{D1}]12 \) and one at 30D. This result suggests that the tight \( ovo^{D1} \) DFS phenotype of \( P[ovo^{D1}]13X13 \) is the result of two \( P[ovo^{D1}] \) insertions. Due to the low frequency of \( P[ovo^{D1}]12 \) transposition, an additional large-scale mobilization experiment was performed to recover flies with darker eye color insertions. Of 18 lines that were recovered, two of them, \( P[ovo^{D1}]12X0 \) and \( P[ovo^{D1}12X13] \), were associated with a fully expressed \( ovo^{D1} \) DFS phenotype (Table 3). \( P[ovo^{D1}]12X0 \) localized to the same position as that of \( P[ovo^{D1}]12 \) suggesting that it is the result of a local transposition.

From the large scale cross between \( CyO, P[ovo^{D1}]13X13; 2-3, Sb/\) males and \( y w \) females, 4 independent jumps were recovered as \( y w \) males with the \( Sb \) marker and \( w^+ \) eye color. One of these, \( P[ovo^{D1}]12X8 \), showed a fully expressed DFS phenotype. Further efforts to destabilize the three insertions, \( P[ovo^{D1}]12, P[ovo^{D1}]16 \) and \( P[ovo^{D1}]15 \), showed that these X-linked insertions could not be mobilized.

From Round 3 transpositions: to recover additional insertions on the third chromosome, we mobilized \( P[ovo^{D1}]31 \) on the \( CyO \) balancer chromosome. From the large scale cross between \( CyO, P[ovo^{D1}]31X4; 2-3, Sb/\) males with \( y w \) females, new insertions were recovered among the progeny as males that segregate the \( w^+ \) eye color from the \( CyO \) chromosome. From 28 lines recovered, 6 segregated with the third chromosome and expressed the DFS \( ovo^{D1} \) phenotype. Germ-line clonal analysis of 144 was performed using 5 of these 6 insertions to determine on which arm they resided. In situ hybridization was performed for 3 of these 6 insertions to determine their locations. One of them, \( P[ovo^{D1}]13X3 \), mapped to position 98A-B and was selected for further analyses since it is associated with an expressed \( P[ovo^{D1}] \) DFS phenotype (1.7% leakiness; Table 3).

Round 4 transpositions: a final set of transpositions was undertaken to recover derivatives of \( P[ovo^{D1}]13X3 \) that fully express the DFS \( ovo^{D1} \) phenotype. From large scale crosses between \( y w/Y; P[ovo^{D1}]13X3; 2-3, Sb \) males and \( y w \) females, more than 25 lines of \( Sb^+ \) males with darker eye colors were recovered. Two of these lines, \( P[ovo^{D1}]13X2 \) and \( P[ovo^{D1}]13X3 \), showed the fully expressed \( ovo^{D1} \) DFS phenotype (Table 3).
Table 4. Frequency of females with germ-line clones generated using the autosomal DFSE technique

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Location</th>
<th>(P[^{ovo}\text{D}1])</th>
<th>#Females</th>
<th>#GLC</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toll^{1444}</td>
<td>97D</td>
<td>C71^*</td>
<td>ND</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>SoxX122</td>
<td>34D</td>
<td>13^†</td>
<td>1060</td>
<td>12</td>
<td>1.13</td>
</tr>
<tr>
<td>rho5A</td>
<td>62A</td>
<td>2X48</td>
<td>1302</td>
<td>19</td>
<td>1.46</td>
</tr>
<tr>
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<td></td>
<td>470</td>
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<td>0.70</td>
</tr>
<tr>
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<td></td>
<td>850</td>
<td>11</td>
<td>1.29</td>
</tr>
<tr>
<td>Gap7B2</td>
<td>67D</td>
<td>2X48</td>
<td>368</td>
<td>6</td>
<td>1.63</td>
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<td>Gap7C4</td>
<td></td>
<td></td>
<td>799</td>
<td>12</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\#Females: number of mutant \(P[^{ovo}\text{D}1]\) females examined.

\#GLC: number of mutant \(P[^{ovo}\text{D}1]\) females with germ-line clones.

Percentage: \(\frac{\text{#GLC}}{\text{#Females}}\) x 100.

ND: the number of females was not determined in this experiment.

*Germ-line clonal analysis of Tollo^{1444} was performed by using \(P[^{ovo}\text{D}1]\) females, as described for \(SosX122\) and \(ovo^{5A}\) (Table 3). Eggs not derived from homzygous germ-line clones have two fused dorsal appendages (Table 3).

† \(P[^{ovo}\text{D}1]\) was used as the DFS mutation for the analysis of \(SosX122\) and \(ovo^{5A}\) was introduced onto the X-chromosome to ensure full expressivity of \(P[^{ovo}\text{D}1]\). The germ-line clone phenotype of \(SosX122\) was described by Lu et al. (1993).

‡ \(P[^{ovo}\text{D}1]\) shows a fully expressed DFS phenotype (Table 2). It was used directly as a DFS without introducing the \(P[^{ovo}\text{D}1]\) mutation on the X-chromosome. The \(rho5A\) germ-line clone phenotype was described by Ruohola-Baker et al. (1993). Like \(rho5A\), neither \(rho5A3\) nor \(rho5A5\) function is required in the germ line for oogenesis (Ruohola-Baker et al. unpublished data).

We also used fully expressed \(P[^{ovo}\text{D}1]\) insertions on 2L and 2R to analyze the maternal effect of different Enhancer of seventeen mutations (Simon et al., 1991). \(P[^{ovo}\text{D}1]\) was used in the analysis of various \(Sos\) alleles and \(P[^{ovo}\text{D}1]\) on the 2L arm, and \(P[^{ovo}\text{D}1]\) on the 2R arm, for further transpositions (Table 2). Approximately 5% of females heterozygous for these two \(P[^{ovo}\text{D}1]\) insertions display a leaky DFS phenotype and lay some eggs. In the presence of only one copy of the wild-type \(ovo\) gene, the leakiness of \(P[^{ovo}\text{D}1]\) and \(P[^{ovo}\text{D}1]\) is reduced significantly (Table 3).

Isolation of insertions that exhibit a fully expressed DFS phenotype

In addition to the new insertions described above, we also recovered in the same experiment a large number of flies associated with darker eye colors, which segregated with the original \(P[^{ovo}\text{D}1]\) chromosome. A darker eye color can be recovered when multiple copies of the transposon are present or when the P-element is inserted into some chromosomal regions that provide high expression of the transgene. More than 80 of these insertions segregated with the original \(P[^{ovo}\text{D}1]\) third chromosome. The female sterility of 10 of these lines was examined in detail and one of them \(P[^{ovo}\text{D}1]\) was found to exhibit a complete DFS sterility similar to that of females heterozygous for \(ovo^{5A}\) (Table 3). Therefore, it is possible that the \(P[^{ovo}\text{D}1]\) line carries either a single insertion in a chromosomal site that increases the expression of the inserted gene or multiple insertions that will increase the amount of the \(ovo^{5A}\) gene product. We performed in situ hybridization to determine whether this chromosome contains multiple insertions. A single site was detected at 70B-C, a position similar to the location of \(P[^{ovo}\text{D}1]\), therefore not allowing us to distinguish between these two possibilities (see Discussion).

Recovery of other autosomal \(P[^{ovo}\text{D}1]\) insertions

Transposition schemes (Fig. 2) similar to the one previously described for \(P[^{ovo}\text{D}1]\) transposition were used to recover insertions on the 3R arm. Because insertions on the X-chromosome did not show any sign of transposition, we used \(P[^{ovo}\text{D}1]\) and \(P[^{ovo}\text{D}1]\) on the 2nd chromosome to start a series of transpositions. One insertion, \(P[^{ovo}\text{D}1]\), that localized to position 98A-B was selected as the 3R \(P[^{ovo}\text{D}1]\). As observed with the 2nd chromosomal insertions, females that carry this insertion sometimes laid eggs (Table 3).

To recover fully expressed \(P[^{ovo}\text{D}1]\) insertions located on 2L, 2R, and 3R, we selected transposition events that were associated with darker eye colors. As expected from the experiments that led to the recovery of the 3L \(P[^{ovo}\text{D}1]\) insertion, these screens allowed us to recover insertions that were associated with a fully expressed DFS phenotype: \(P[^{ovo}\text{D}1]\) on 2L, \(P[^{ovo}\text{D}1]\) on 3R, and \(P[^{ovo}\text{D}1]\) on 3R. Females heterozygous for these insertions do not lay eggs (Table 3).

In conclusion, through a series of transpositions, starting with \(P[^{ovo}\text{D}1]\), we have recovered \(P[^{ovo}\text{D}1]\) insertions on each autosomal arm which are associated with fully expressed DFS phenotypes that are similar to the original \(ovo^{5A}\) X-linked mutation. We hence designate \(P[^{ovo}\text{D}1]\) as \(P[^{ovo}\text{D}1]\), \(P[^{ovo}\text{D}1]\), \(P[^{ovo}\text{D}1]\), \(P[^{ovo}\text{D}1]\), \(P[^{ovo}\text{D}1]\), \(P[^{ovo}\text{D}1]\), and \(P[^{ovo}\text{D}1]\) as \(P[^{ovo}\text{D}1]\).

The germ-line dependence of autosomal \(P[^{ovo}\text{D}1]\) insertions

The sterility of \(ovo^{5A}\) is a germ-line-dependent phenomenon (Busson et al., 1983; Perrimon and Gans, 1983; Perrimon, 1984), and we expected that the autosomal \(P[^{ovo}\text{D}1]\) insertions that we recovered would display the same germ-line dependence. To test this, we examined the eggs derived from females of genotype \(P[^{ovo}\text{D}1]\) / \(Tr^{444}\). X-ray irradiated as larvae. \(Tr^{444}\) is a loss-of-function \(Toll\) allele, which produces dorsalized embryos as a recessive maternal effect (Anderson et al., 1985). This maternal effect is strictly due to the germ-line-dependent expression of \(Tl\) (Schubach and Wieschaus, 1986). We used \(P[^{ovo}\text{D}1]\) as a DFS mutation to generate \(Tr^{444}\) homozygous germ-line clones. As expected, fertilized eggs derived from these females were all dorsalized (data not shown).

The frequency of mosaics recovered using these \(P[^{ovo}\text{D}1]\) insertions was determined following a mosaic analysis of several zygotic lethal mutations (Table 4). We found that...
approximately 1-2% of heterozygote females irradiated at the end of the first larval instar stage (see Materials and Methods) carried a germ-line clone. Such frequencies of germ-line mosaicism are similar to those reported previously using $Fs(2)D$ and $Fs(2)I$ (MacDonald and Struhl, 1986; Wieschaus et al., 1984; N. P. unpublished).

**Germ-line clonal analysis of Gap1 gene**

Components of the p21ras signaling pathway have been implicated in the signaling of many receptor protein tyrosine kinases such as sevenless and Torso (reviewed in Perrimon, 1993). Both Ras1 and Sos, an exchange factor encoded by Son of sevenless, have been shown to operate in signaling of both Torso (Lu et al., 1993; Doyle and Bishop, 1993) and Sevenless (Rogge et al., 1991; Simon et al., 1991; Bonfini et al., 1992). To determine whether Gap-1, which encodes a Ras-GTPase-activating protein implicated in Sevenless signaling (Gaul et al., 1992), also operates in Torso signaling, we examined the maternal effect phenotype of two Gap1 mutations, Gap1$mipw^+$ and Gap1$B2^+$ (Buckles et al., 1992) and Gap1$B2^+$ (Gaul et al., 1992). Homozygous Gap1$mipw^+$ and Gap1$B2^+$ females lay eggs that show a weak dorsalized egg shell morphology (Fig. 3B). The base of the appendages is expanded towards the ventral side of the egg (Fig. 3B). In addition, embryos derived from Gap1 homzygous females are partially dorsalized (Fig. 3D). Since the weak dorsalization phenotype of both the egg and the embryo may result from Gap1 gene function in either somatic follicle cells or germ-line cells, we decided to analyze the tissue specificity of Gap1 mutations. If the combination of mutant germ line and wild-type soma leads to the mutant phenotype, then the function of this gene is necessary in the germ line. On the other hand, if under these conditions wild-type instead of the mutant phenotype is produced, then the mutation is due to gene function in the somatic follicle tissue. When crossed to either heterozygous Gap1 or wild-type males, all 6 Gap1$B2^+$

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**Fig. 3.** Somatic dependence of Gap1 mutations. Two alleles at the Gap1 locus, mipw$^+$ and Gap1$B2^+$ were used in this analysis. mipw$^+$ is denoted as Gap1$mipw^+$ since mip and Gap1 are allelic (Buckles et al., 1992; Gaul et al., 1992). While Gap1$mipw^+$ is a strong allele of the mip gene (Buckles et al., 1992), Gap1$B2^+$ is more likely to represent a complete loss-of-function mutation (Gaul et al., 1992). Both Gap1$mipw^+$ and Gap1$B2^+$ homozygous females are associated with semi-sterility. When crossed to wild-type males approximatively 80% of the eggs do not hatch. (A) Wild-type egg. (B) Weak dorsalized egg laid by Gap1$B2^+$ females. The basal appendage material is enlarged towards the ventral side of the egg. The degree of dorsalization of Gap1$mipw^+$ eggs is slightly weaker than in Gap1$B2^+$ eggs. (C) Wild-type embryo. (D) Weak partially dorsalized embryo produced by Gap1$mipw^+$ females. Variability in the degree of dorsalization is apparent in embryos derived from Gap1$mipw^+$ and Gap1$B2^+$ homozygous females. In some cases, embryos were completely dorsalized (data not shown).
and 11 out of 12 Gap1\textsuperscript{mipw+} mosaic females showed no maternal effect. Eggs with wild-type chorion morphology were laid and larvae hatched. Some of the larvae died during early larval stages without obvious cuticle defects. One Gap1\textsuperscript{mipw+} mosaic female laid a few embryos showing a maternal effect lethal phenotype, which in no case resembled dorsalized embryos (data not shown).

We conclude that the weak dorsalization of both the egg shell and embryo are not germ-line dependent and Gap1 function in follicle cells is required for proper egg shell and embryonic dorsal-ventral polarity.

**DISCUSSION**

To extend the DFS technique to the autosomes, we have cloned the \textit{ovoD1} mutation into a P-element vector and recovered fully expressed \textit{P[ovoD1]} insertions on each autosomal arm. Using the autosomal DFS technique, we have analyzed the germ-line clone phenotypes of several zygotic lethal mutations. This analysis indicates that the DFS sterility of \textit{P[ovoD1]} insertions is germ-line dependent and that these \textit{P[ovoD1]} insertions are excellent tools for generating germ-line chimeras.

**Incomplete expressivity of \textit{P[ovoD1]} insertions**

Surprisingly, the 18 kb \textit{P[ovoD1]} insertions that contain 8 kb of upstream and 3kb of downstream DNA along with the counterpart of the 7.2 kb \textit{ovo\textsuperscript{+}} rescuing fragment showed a weak \textit{ovoD1} DFS phenotype. We tested more than 100 lines that carried a newly-inserted \textit{P[ovoD1]} and did not recover an insertion associated with complete expressivity. This suggests that the \textit{ovo} gene may be larger and more complex than originally proposed (Mevel-Ninio et al., 1991; Garfinkel et al., 1992). It is only following local transposition that we were able to recover \textit{P[ovoD1]} insertions that had similar genetic properties to the X-linked \textit{ovoD1} mutation.

We envision two possibilities for the production of tight \textit{P[ovoD1]} insertions. First, they may have been generated by duplication of the original insertion. Precedents for such duplication events have been reported previously (Rotha et al., 1988; Salz et al., 1987; Hawley et al., 1988). Increased expressivity of the DFS phenotype is most likely achieved by expressing two copies of \textit{P[ovoD1]} so that two copies of the wild-type X-linked \textit{ovo} gene are fully antagonized. With the exception of \textit{P[ovoD1]}\textsuperscript{Sx13}, only one in situ signal was detectable in all tight \textit{P[ovoD1]} insertions examined. Recently it was reported that P-elements preferentially transpose into the genomic region very close to their original insertion sites (Tower et al., 1993; Zhang and Spradling, 1993). These observations suggest that duplication of the P-element transposon may have occurred locally in the \textit{P[ovoD1]} lines that are associated with a darker eye color and full DFS expressivity as the result of the expression of two copies of \textit{P[ovoD1]}. Another alternative is that following local transposition, higher levels of \textit{P[ovoD1]} expression from the new insertion site. Further characterization of \textit{P[ovoD1]} insertions that display either strong or weak DFS phenotypes will be needed to resolve this issue.

**Gap1 gene is required in the follicle cells for dorsoventral polarity**

During oogenesis, signaling between the oocyte and follicle cells lead to the asymmetric differentiation of the follicle cells along the dorsal-ventral axis (reviewed in Schupbach et al., 1991; Govind and Steward, 1991). Mutations that disrupt these processes alter the polarity of both the egg shell and the embryo. It has been proposed that a dorsalizing signal is sent from the germ-line vesicle/oocyte nucleus to the surrounding dorsal follicle cells and induces a dorsal cell fate (Schupbach, 1987; Manseau and Schupbach, 1989; Kelly, 1993; Ruohola-Baker et al., 1993). This signal may serve as the ligand for Torpedo, which is a \textit{Drosophila} EGF-receptor homologue functioning in the soma (Schupbach, 1987; Price et al., 1989; Schejter and Shilo, 1989; Clifford and Schupbach, 1989). Up to now, components that might transduce signals from activated Torpedo have not yet been characterized.

Different receptor tyrosine kinases use common intracellular signaling components that include \textit{Sos}, \textit{Ras1}, and \textit{drk} (Simon et al., 1993; Olivier et al., 1993; Lu et al., 1993; Doyle and Bishop, 1993; Sturtevant et al., 1993; Perrimon, 1993). Our germ-line clonal analysis of \textit{Gap1} mutations suggests that \textit{Gap1} acts in the establishment of dorsal/ventral polarity of the egg chamber. Based on these observations we propose that components of the Gap1/Ras1 pathway function in transducing the signal from an activated Torpedo receptor tyrosine kinase. In this model \textit{Gap1} loss-of-function mutations will give rise to dorsalized egg shell and embryo by increasing the level of Ras-GTP in the follicle cells.

Since the two \textit{Gap1} mutations we tested in our germ-line clone analysis are not associated with a germ-line-dependent maternal effect, it suggests that \textit{Gap1} is not involved in Torso signaling. This result suggests the presence of at least a second Ras-GAP \textit{Drosophila} protein. However, a formal possibility is that the two \textit{Gap1} mutations we tested are not complete null mutations. Further work will be required to clarify this issue.

**The autosomal FLP-DFS technique**

In this paper, we have described the recovery of \textit{P[ovoD1]} insertions that allow the easy generation of germ-line mosaics for autosomal mutations. However, the frequency of X-ray induced germ-line clone induction is low. On the X-chromosome, approximately 5% of chimeras can be recovered in females heterozygous for \textit{ovoD1} (Perrimon, 1984). On the autosomes, a lower frequency of only 1 to 2% was achieved using \textit{P[ovoD1]} insertions as DFS mutations.

To increase the efficiency of germ-line mosaic production, we previously developed the ‘FLP-DFS’ technique (Chou and Perrimon, 1992). This technique relies on the ability of the yeast FLP-recombinase to catalyze and promote mitotic exchange between chromosomes specifically at FRT (FLP-Recombinase Target) sites (Golic, 1991). In this method, the site of recombination is determined by the position of the FRT sequences and the efficiency of mitotic recombination is controlled by the heat shock inducible FLP-recombinase. We have shown that greater than 90% of germ-line mosaics can be easily recovered in
heterozygous ovo^{D1} females following heat shock induction during larval stage.

The availability of the autosomal P[ovo^{D1}] insertions with similar genetic properties to the ovo^{D1} mutation will allow us to extend this ‘FLP-DFS’ technique to the autosomes. The construction of chromosomes that carry FRT elements located at the base of each chromosome with a proximal P[ovo^{D1}] insertion will provide the tools necessary for large scale analyses of the maternal effects of essential genes on the autosomes.

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that the sans fille locus is involved in Drosophila sex determination. *Genetics* **120**, 159-171.


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