Requirement for Cell-Proliferation Control Genes in Drosophila Oogenesis

Janos Szabad,∗,† Victoria A. Jursnich†,* and Peter J. Bryant†

†Howard Hughes Medical Institute, Department of Biology, University of Utah, Salt Lake City, Utah 84112 and Institute of Genetics, Biological Research Center, H-6701 Szeged, P.O. Box 521, Hungary, and Developmental Biology Center, University of California, Irvine, California 92717

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ABSTRACT

Genes that are required for cell proliferation control in Drosophila imaginal discs were tested for function in the female germ-line and follicle cells. Chimeras and mosaics were produced in which developing oocytes and nurse cells were mutant at one of five imaginal disc overgrowth loci (fat, lgd, lgl, c43 and dco) while the enveloping follicle cells were normal. The chimeras were produced by transplantation of pole cells and the mosaics were produced by X-ray-induced mitotic recombination using the dominant female-sterile technique. The results show that each of the genes tested plays an essential role in the development or function of the female germ line. The fat, lgl and c43 homozygous germ-line clones fail to produce eggs, indicating a germ-line requirement for the corresponding genes. Perdurance of the fat* gene product in mitotic recombination clones allows the formation of a few infertile eggs from fat homozygous germ-line cells. The lgd homozygous germ-line clones give rise to a few eggs with abnormal chorionic appendages, a defect thought to result from defective cell communication between the mutant germ-line and the nonmutant follicle cells. One allele of dco (dco118) prevents egg development when homozygous in the germ line, whereas the dco118 allele has no effect on germ-line development. F2(ku1gra), a recently described follicle cell-dependent dominant female-sterile mutation, allowed the analysis of egg primordia in which fat, lgl or lgd homozygous mutant follicle cells surrounded normal oocytes. The results show that the fat and lgd genes are not required for follicle cell functions, while absence of lgl function in follicles prevents egg development. Whereas the products of these genes are necessary for the cell interactions that control cell proliferation in imaginal discs, they may be needed for cell interactions that control other aspects of development in the ovary.

The analysis of lethal mutations leading to imaginal disc overgrowth in Drosophila has resulted in the identification of seven genes that act as negative regulators of cell proliferation in these tissues (Bryant and Schmidt 1991). Mutations in two of these genes [dlg; Stewart, Murphy and Fristrom (1972) and lgl; Gateff and Schneiderman (1974)] lead to neoplastic overgrowth, in which the imaginal disc loses its single-layered epithelial structure and its ability to differentiate. Mutations at the other five loci [c43, Martin, Martin and Shearn (1977); dco, Jursnich et al. (1990); fat, Bryant et al. (1988); lgd, Bryant and Schubiger (1971); and tud, Gateff and Mechler (1989)] lead to hyperplastic overgrowth, in which the tissue retains its single-layered epithelial structure and ability to differentiate. In all cases the disc overgrowth occurs during an extended larval period, and the animal dies either as a larva or as a pupa. Although imaginal disc overgrowth is the most conspicuous aspect of these phenotypes, other cell populations are also sometimes affected. The larval brain overgrows in lgl (Gateff and Schneiderman 1974) anddlg (Woods and Bryant 1989), and the imaginal primordia of various adult structures overgrow in lgd (Bryant and Levinson, 1985). lgl and lgd cause larval bloating (Gateff and Schneiderman 1974; Woods and Bryant 1989) and some alleles of dco cause slow growth and larval lethality (Jursnich et al. 1990), indicating that cell populations other than imaginal discs are affected.

In this paper we investigate the requirement for cell-proliferation control genes in the ovary, which contains two different proliferating cell types—the germ line and the mesodermally derived follicle cells. Both cell types show characteristic proliferation patterns in normal flies (Mahowald and Kambselles 1980) and therefore might require the function of cell-proliferation control genes. Six genes which function in proliferation control in the germ cells of the ovary have been identified by the finding of gonial cell tumors in mutant females (Gateff 1982; King and Storto 1988). In one case, mutations lead to gonial cell tumors in both males and females (Gateff and Mechler 1989). The molecular basis for these functions has not yet been analyzed.

Several pieces of evidence suggest that excessive cell
proliferation in the imaginal disc overgrowth mutants is a consequence of defective interaction between neighboring cells of the disc (BRYANT 1987; LÜTZELSCHWAB et al. 1987; PERRIMON 1988; KLÄMBT et al. 1989). The lgl gene has been shown to encode a cell-surface molecule with some similarities to calcium-dependent cell adhesion molecules (KLÄMBT et al. 1989), consistent with a requirement for this gene in cell interaction. In dco and c43 imaginal discs there is a dramatic reduction of dye coupling between cells, indicating defects in gap-junctional cell communication (JURSNICH et al. 1990), and in c43 and some dco genotypes there is also a reduced density of gap junctions between imaginal disc cells (RYERSE and NAGEL 1984; JURSNICH et al. 1990).

In the ovary there are extensive interactions between the 16 cells of the cystocyte cluster leading to the determination of one of these cells as the oocyte and the remainder as nurse cells (MAHOWALD and KAMBSSELLIS 1980). Subsequently, interactions take the form of transfer of cytoplasm from the nurse cells to the oocyte. Interactions between the oocyte and follicle cells lead to the deposition of chorion by those follicle cells that contact the oocyte, and the form of the chorion is apparently controlled not only by the follicle cells but also by interactions between the germ-line and the follicle cells. Genetic studies confirm that cell communication between the germ-line and the enveloping follicle cells plays an important role in the determination of one of these cells as the oocyte (WIESCHAUS, MARSH and SCHUBIGER 1971; P. BRYANT and J. SZIDONYA, unpublished). Genetic studies confirm that cell communication between the germ-line and the enveloping follicle cells plays an important role in the determination of one of these cells as the oocyte (WIESCHAUS, MARSH and SCHUBIGER 1971; P. BRYANT and J. SZIDONYA, unpublished). Genetic studies confirm that cell communication between the germ-line and the enveloping follicle cells plays an important role in the determination of one of these cells as the oocyte (WIESCHAUS, MARSH and SCHUBIGER 1971; P. BRYANT and J. SZIDONYA, unpublished).

MATERIALS AND METHODS

Imaginal disc overgrowth mutations: The following imaginal disc overgrowth mutations were studied (the specific alleles used are indicated in the RESULTS): lethal(2)fat (fat; located at cytotgenetic position 24D5.6–7; BRYANT et al. 1988); lethal(2)giant discs (lgd; located at 32D1–4; BRYANT and SCHÜBGER 1971; P. BRYANT and J. SZIDONYA, unpublished); lethal(2)giant larvae (lgl; located at 21A; GATEFF and SCHNEIDERMAN 1974), lethal(3)c43 (c43; located at 85E; MARTIN, MARTIN and SHEARN 1977) and lethal(3)discs overgrown (dco; located at 100A5.6–100B1.2; JURSNICH et al. 1990). All of the mutations are recessive zygotic lethals and are kept in balanced stocks. The chromosomes carrying the mutations (except lgl) are labeled with recessive marker mutations (Table 1; LINDSLEY and GRELL 1968). All the experiments were performed at 25°C, except the test involving the temperature-sensitive mutation c43 which was performed at 29°C.

Production of germ-line chimeras by pole-cell transplantation: Germ-line chimeras for fat, lgd, c43 and dco were constructed by transplanting mutant pole cells into nonmutant hosts (LEHMANN and NÜSSLIN-VOGEL 1986). In most cases, the mutant pole cells were obtained from embryos derived from a cross between parents carrying different mutant alleles of the gene or by using recombinant chromosomes in which all but the region containing the relevant mutation has been replaced by lethal-fraction chromosome segments (Table 1). This was done in order to avoid the effects of possibly unrecognized deleterious second-site recessive mutations on the mutant chromosomes. This was not necessary for c43 because this mutation is homozygous viable and fertile at the permissive temperature (18–20°C), and the chromosome carrying it is therefore probably free of second-site mutations affecting viability and fertility.

Different balancer chromosomes (balancer+ and balancerex) which are not lethal in heterozygous combination, were used in the two parents of donor embryos (Table 1). This was done to avoid the production of donor embryos homozygous for balancer chromosomes, since pole cells from such embryos can produce a mutant egg phenotype which could be confused with that caused by the overgrowth mutation (TAUBERT and SZABAD 1987). The lethal/balancer as well as the balancer+balancerex germ-line chimeras served as internal controls.

Host embryos were derived from a cross between wild-type (OreR) females and Fs(1)K1237/Y (=ovoD) males. Fs(1)K1237 (=K1237) is a strictly germ-line-dependent dominant female-sterile mutation (KOMITOPOLOU et al. 1983; BUSSON et al. 1985), which blocks the development of egg primordia around the beginning of vitellogenesis. After eclosion, the +/K1237 females were mated with a px or males in the experiments with fat and lgd, and with cu red e males in the tests of c43 and dco. This made it possible to determine, in retrospect, the genotype of the transplanted pole cells (see Table 1). Egg production of the chimeras was followed throughout a test period of 12–14 days.

Production of germ-line mosaics by mitotic recombination: Germ-line clones homozygous for fat18, lgd55 or lg18 were generated by mitotic recombination using the dominant female-sterile technique (WIESCHAUS 1980; PERRIMON and GANS 1983). This technique could not be used for the third-chromosome mutations because an appropriate dominant female-sterile mutation is not available. fat18 dpovo or Fs(2)1, lgd55 dpovo or Fs(2)1, and lg18/Fs(2)1 larvae were irradiated at three developmental stages with 1000R of X-rays for the induction of mitotic recombination. The eclosing females were collected as virgins and mated with dpovo or (in the cases of fat and lgd) wild type (in the case of lgl) and tested for ten days to identify most (>95%) of the mosiacs (WIESCHAUS and SZABAD 1979). The mosiacs were tested for five additional days. Fs(2)1 is a germ line-dependent dominant female-sterile mutation (SZABAD, ERDELY, and SZIDONYA 1987). It is located on the left arm of the second chromosome, distal to the fat and lgd loci. In the absence of mitotic recombination, the Fs(2)1-carrying females deposit...
very few eggs, and these are rudimentary, needle-shaped, without dorsal chorionic appendages, and usually flaccid (Figure 1c).

Most of the mitotic recombination events on the left arm of the second chromosome in these flies lead to the formation of fat"/dp<sup>nn</sup>, lg<sup>de</sup>/dp<sup>nn</sup>, or lg<sup>h</sup> homozygous germ-line cells. These cells are free from the effects of Fs(2)I and may continue development unless homozygosity for the disc overgrowth mutation interferes with that process. Homozygosity for dp<sup>nn</sup> is not expected to have any effect on egg development since dp<sup>nn</sup> homozygous females are viable and fertile. Mitotic recombination may occasionally take place between the fat (or lgd) and Fs(2)I loci. In these cases, the Fs(2)I-free cell will be heterozygous for fat<sup>de</sup> (or lg<sup>de</sup>) and dp<sup>nn</sup>, and is expected to produce offspring; these uninformative events were recognized by the phenotype of the progeny.

**Production of follicle cell mosaics by mitotic recombination:** Follicle-cell clones homozygous for fat<sup>de</sup>, lg<sup>de</sup> or lg<sup>h</sup> were generated by mitotic recombination using a new variation of the dominant female-sterile technique. fat<sup>de</sup> dp<sup>nn</sup> or (Fs(2)I/uga, lgd<sup>de</sup> dp<sup>nn</sup> or (Fs(2)I/uga, or lg<sup>h</sup>)(Fs(2)I/uga larvae were irradiated with 1000 R of X-rays. The eclosing females were mated with bw males (the Fs(2)I/uga chromosome is marked with bw) and tested for 14 days in order to identify at least 95% of the mosaic (SZABAD et al. 1989). Fs(2)I/uga is a follicle-cell-dependent dominant female-sterile mutation located at 2-18. In the absence of mitotic recombination, the Fs(2)I/uga-carrying females do not deposit eggs. However, following mitotic recombination Fs(2)I/uga-free follicle cells may be formed which will support egg development unless homozygosity for the imaginal disc overgrowth mutation interferes with their function.

**Analysis of phenotypes:** Females that did not deposit eggs during the test period were dissected and examined for the presence of vitellogenic egg chambers or abnormal ovarioles or egg chambers. Unhatched eggs and embryos were prepared for microscopy by mounting in Hoyer's solution (WIESCHAUS and NÜSSLLEN-VOLHARD 1986). Larval phenotypes were analyzed by dissection and observation under a dissecting microscope. Imaginal discs were dissected out in Ringer's solution and photographed under a compound microscope.

**RESULTS**

**Effects of fat on germ-line development:** The fat product is required in the germ line for normal egg development and/or subsequent embryogenesis. When pole cells of embryos from fat heterozygous parents were transplanted into +/K1237 hosts no fat/ fat germ-line chimeras were recovered even though about four were expected based on the numbers of control chimeras (P < 0.05; y<sup>2</sup> test) (Table 1). All 97 embryos examined from the above cross were found to have pole cells, indicating their presence in the heteroallelic mutant embryos. None of the 28 host females that failed to deposit eggs showed vitellogenic egg primordia, indicating that egg chambers with heteroallelic fat germ-line cells surrounded by fat<sup>+</sup> somatic cells do not develop to vitellogenic stages.

The effects of the fat mutation were also analyzed in clones induced by mitotic recombination. Only one of the 440 fat<sup>de</sup> dp<sup>nn</sup> /Fs(2)I females, irradiated as first-instar larvae, gave rise to a single, apparently normal egg (Table 2). The Fs(2)I-free clones appeared with frequencies similar to the controls when second and young third-instar larvae (48-68 hr and 68-78 hr, respectively) were irradiated but the number of eggs produced was significantly smaller than controls (Table 2). All eggs derived from fat<sup>de</sup> clones were apparently normal, but the resulting embryos did not develop to the stage of cuticle formation. The females that contained fat homozygous clones deposited all of their eggs during the first four days of adulthood, whereas the control mosaics deposited eggs throughout their lives.

**Effects of lgd on germ-line development:** The lgd gene product is required for normal germ-line development and/or subsequent embryogenesis. Transplantation of pole cells from embryos produced by lgd heterozygous parents resulted in eight chimeras, three of which probably carried lgd homozygous germ cells (Table 1). These chimeras produced mostly abnormal eggs at a uniform rate throughout the 14-day test period. About half of these eggs were flaccid and slightly reduced in size. The dorsal appendages were often fused at the site of their origin and were wider than in wild type (Figure 1b). A few of the eggs did not collapse and embryos developed in several of them, although the dorsal appendages were still slightly abnormal. Most of the developing larvae died prior to hatching. A few of the larvae appeared normal, but others showed severe head defects and in these cases the ventral setal belts were often narrower than in wild type and sometimes fused or missing as in the cases illustrated by MAYER and NUSSLEIN-VOLHARD (1988). Antennal and maxillary sense organs and Keilin's organs were missing or abnormal, and the head skeleton was reduced or absent in almost all cases. In two cases, larvae hatched and developed to pharate adults without any apparent defects. The three chimeras with mutant germ cells produced only 0.7 ± 0.4 eggs per day, compared with their sibling chimeras which deposited 23 ± 15.7 eggs per day. Several of their vitellogenic egg primordia showed degeneration and many of the developing egg chambers contained more than 40 cells (as compared to the usual 15 nurse cells), a feature characteristic of the ovarian tumor mutants (KING and STORTO 1988).

Mitotic recombination gave lgd<sup>de</sup> homozygous germ-line clones at frequencies similar to the controls, but the number of eggs produced was only about 4% of the control number (Table 2). The lgd<sup>de</sup>-derived eggs had fused and reduced dorsal appendages (Figure 1d), but the site of origin of the appendages and the chorion cell imprint was not noticeably abnormal (cf. SCHÜPBACH 1987). The embryos were similar to those derived from lgd chimeras.

**Effects of Igl on germ-line development:** Our re-
Fertility of chimeras produced by transplantation of mutant pole cells into Fs(1)1237 hosts

<table>
<thead>
<tr>
<th>Locus</th>
<th>Parents of donor embryos*</th>
<th>Parents of host embryos</th>
<th>Male partners of host females</th>
<th>No. of fertile chimeras^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fat</td>
<td>fat^16</td>
<td>dp^en or/FyO</td>
<td>fat^13//Fc Gla</td>
<td>+/+</td>
</tr>
<tr>
<td>lqd</td>
<td>lqd^1</td>
<td>a px or/FyO</td>
<td>lqd^4/ dp^en or/Bc Gla</td>
<td>+/+</td>
</tr>
<tr>
<td>c43</td>
<td>c43 red/TM3</td>
<td></td>
<td>c43 red//CxD</td>
<td>+/+</td>
</tr>
<tr>
<td>dco</td>
<td>dco^15</td>
<td>ri//TM6</td>
<td>dco^12 cu//TM3</td>
<td>+/+</td>
</tr>
<tr>
<td>dpoWN</td>
<td>or/Fs(P)I</td>
<td></td>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>dpoW</td>
<td>or/Fs(P)I</td>
<td></td>
<td></td>
<td>+/+</td>
</tr>
</tbody>
</table>

*CyO, TM3, TM6, Bc Gla and CxD are balancer chromosomes (LINDSLEY and GRELL 1968). dco^-, the Dp(3;1)48; Df(3R)A113 synthetic deficiency that lacks dco (JURSNICH et al. 1990). ^Genotype of transplanted pole cells deduced from progeny. Bal, chromosome labeled with dominant marker mutations.

Effect of fat, lqd and lgl on female germ-line cells in genetic mosaics

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age at irradiation^2</th>
<th>Tested</th>
<th>Mosaic</th>
<th>%</th>
<th>Eggs/day/mosaic^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28-48</td>
<td>164</td>
<td>11</td>
<td>6.7</td>
<td>6.7 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>48-68</td>
<td>360</td>
<td>16</td>
<td>4.4</td>
<td>5.6 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>68-78</td>
<td>378</td>
<td>10</td>
<td>2.6</td>
<td>1.6 ± 1.3</td>
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<tr>
<td>fat^16</td>
<td>28-48</td>
<td>440</td>
<td>1</td>
<td>0.2**</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>48-68</td>
<td>219</td>
<td>7^*</td>
<td>2.0</td>
<td>0.22 ± 0.09**</td>
</tr>
<tr>
<td></td>
<td>68-78</td>
<td>171</td>
<td>6^*</td>
<td>5.5</td>
<td>0.43 ± 0.21*</td>
</tr>
<tr>
<td>lqd^4</td>
<td>28-48</td>
<td>75</td>
<td>4</td>
<td>5.0</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>48-68</td>
<td>80</td>
<td>6</td>
<td>7.5</td>
<td>0.23 ± 0.17**</td>
</tr>
<tr>
<td>lgl^1</td>
<td>28-48</td>
<td>121</td>
<td>0</td>
<td></td>
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<tr>
<td></td>
<td>48-68</td>
<td>108</td>
<td>1^*</td>
<td>0.93</td>
<td>5.1 ± 4.6</td>
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<tr>
<td></td>
<td>68-78</td>
<td>173</td>
<td>0</td>
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</table>

*fat^16 dp^en or/Fs(2)1 or lqd^4/ dp^en or/Fs(2)1 and, as control, dp^en or/Fs(2)1 larvae were irradiated by 1000 R of X-rays (250 kv; 3-mm Al filter; 500 R/min). ^Hours after the initiation of development; 28-48 represents first-instar larvae, 48-68 spans most of the second instar, and 68-78-hr-old larvae are late second/early third instars. +Mosaic females were screened for 15 days. 4One of the mosaics produced eggs at the control rate. Some dp^ en progeny developed from these mosaics, showing that mitotic recombination had occurred between the dp and Fs(2)1 loe (i.e., distal to fat]) and/or that the Fs(2)1 mutation had reverted. These mosaics were not included in determining the egg production rate. ^This mosaic probably arose through reversion of Fs(2)1 and/or double mitotic recombination. ^* and ** = significantly different from the control at P < 0.05 and 0.01, respectively [see KASTERNAU and BOWMAN (1970) for the frequency of mosaicism and t-test for the rate of egg production].

RESULTS indicate that the lgl gene is required in the germ line for egg development. Approximately eight of the 121 lgl^1//Fs(2)1 females, irradiated as first-instar larvae, should have carried lgl^1 homozygous germ-line clones (Table 2). However, none of the females deposited eggs (Table 2), indicating that the lgl^1 mutation blocks oogenesis when homozygous in the germ line. Similarly, no eggs were produced when the clones were induced in second or early third-instar larvae (Table 2). The single mosaic female that deposited eggs at the control rate must have arisen through reversion of the Fs(2)1 mutation or a rare double mitotic-recombination event in the lgl-Fs(2)1 and the Fs(2)1-centromere intervals as shown by the observation that about 50% of her offspring carried the lgl^1 allele.

Effect of c43 on germ-line development: c43 homozygous germ-line cells do not give rise to vitellogenic egg primordia and therefore do not produce eggs or progeny at the restrictive temperature (29°). Although 3-4 of the 31 host embryos should have received c43 homozygous pole cells in this experiment, no adult chimeras of this type were detected (Table 1; P < 0.05; χ^2 test). Vitellogenic egg primordia were absent from the ovaries of the 31 females that failed to deposit eggs during the 12-day test period.

Effect of dco on germ-line development: Our results indicate that the dco wild-type function is required in the germ line and that this function is impaired by dco^enb but not by the dco^18 mutation. Different homozygous and heteroallelic combinations of dco alleles produce a variety of effects on larval and imaginal disc development; dco^18/dco^18 zygotes show an imaginal disc overgrowth phenotype during a prolonged larval life; dco^18/dco^- zygotes die as embryos, and dco^enb/dco^18 embryos do not die as larvae with a "discless" phenotype (JURSNICH et al. 1990). We have tested each of these genotypes in chimeras, with the following results:

1. In the first experiment, pole cells of embryos from dco^18/TM6B and dco^18/TM3 parents were transplanted into +/K1237 hosts, and two chimeras were recovered carrying dco^18/dco^18 germ-line cells (Table 1). Eggs were produced by these chimeras at approximately the same rate as that of the sibling chimeras carrying dco^18/TM3, dco^18/TM6B or TM3/TM6B germ-line cells (16.9 ± 12.3 versus 21.3 ± 14.0 eggs/chimera/day). Adult progeny developed from virtually all the eggs produced by the dco^18/dco^18 germ-line chimeras. This result shows that the dco^18/dco^18 combination does not interfere with germ-line
functions, or that the necessary dco gene functions are rescued by zygotic activity of the paternally introduced wild-type allele.

2. In the second experiment, pole cell transplantation were done in which some host embryos should have received \textit{dco}^{18}/\textit{dco}^{-} pole cells (Table 1). The eclosing females were mated with \textit{dco}^{18}/TM6 males. Progeny analysis of the chimeras (data not shown) indicated that one of the chimeras carried \textit{dco}^{18}/\textit{dco}^{-} germ-line cells. Besides the TM6-carrying adult offspring, she produced two classes of non-viable progeny. In the first class, the embryos died in the egg case. While tail and abdominal structures of these embryos appeared normal, the head showed variable abnormalities (Figure 2). The least severe cases showed minor abnormalities of the labral structures and dorsal bridge (Figure 2b; cf. JÜRGENS et al., 1986). More severe cases were represented by larvae in which most head components were deleted, but antennal and maxillary sense organs (often abnormal) as well as the mouth hooks and remnants of the head skeleton were still present (Figure 2c). Several of these embryos showed the “tail-up” phenotype, an indication of abnormal gastrulation (KONRAD, GORALSKI and MAHOWALD 1988). In the most severe cases, the head structures were absent (Figure 2d) and a hole appeared on the dorsal larval cuticle, a feature called “dorsal open” and previously described for embryos derived from \textit{lethal(1)discs large-1} germ-line clones (PERRIMON 1988). That the above embryos represent the \textit{dco}^{18}/\textit{dco}^{-} zygotes is confirmed by the observation that almost identical embryos are found among the progeny of \textit{dco}^{18}/+ crossed with \textit{dco}^{-}/+ parents. The second class of nonviable progeny showed polyphasic lethality, with most of the larvae dying around the second-third larval instar transition. They usually did not complete the molt, but showed two sets of mouth hooks, setal belts and filzkörper (Figures 2, e and f). A few of the larvae survived to the third instar, but had small imaginal discs with abnormal folds. These
larvae were indistinguishable from dcoa homozygotes produced by dcoa/+ parents (JURSNICH et al. 1990). The results of this experiment show that the dcoa/dco- female germ-line cells are functional. This could indicate that function of the dco gene is not required for germ-line function, or that the defect caused by the hypomorphic dcoa allele is not sufficient to interfere with a required germ-line function.

3. In the third experiment, pole cells of embryos from dto'' heterozygous parents were transplanted into +/K1237 hosts (Table 1). No dco''/dco- chimeras were identified despite the fact that 4-5 of the host embryos should have received dcoa homozygous pole cells (Table 1). No vitellogenic egg primordia developed in the 44 sterile females, indicating that the dco'' mutation interferes with germ-line development before this stage.

Follicle cell mosaics: The effects of the disc overgrowth mutations fatid, lgd45, and lgl4 on follicle cells were studied by producing follicle cell clones homozygous for each mutation, using the dominant female sterile technique. fatid (or lgd45 or lgl4)/Fs(2)Ugra larvae were irradiated in order to induce fatid (or lgd45 or lgl4) homozygous clones which had lost the Fs(2)Ugra mutation. In the case of fatid and lgd45, mosaic females were recovered with frequencies indistinguishable from the controls (Table 3), and they gave rise to offspring at about the control rate. These results indicate that the fat and lgd genes are not required for follicle cell functions.

Although 9-10 mosaics were expected in the case

### Table 3

<table>
<thead>
<tr>
<th>Females</th>
<th>Mutation</th>
<th>Age at irradiation</th>
<th>Tested</th>
<th>Mosaic</th>
<th>Eggs/day/mosaic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>45-53</td>
<td>254</td>
<td>12</td>
<td>0.068 ± 0.040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53-68</td>
<td>158</td>
<td>6</td>
<td>0.034 ± 0.031</td>
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<tr>
<td>fatid</td>
<td></td>
<td>45-53</td>
<td>459</td>
<td>19</td>
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<td></td>
<td></td>
<td>53-68</td>
<td>438</td>
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<td>0.025 ± 0.014</td>
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<td>lgd45</td>
<td></td>
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<td>0.044 ± 0.031</td>
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<td>53-68</td>
<td>145</td>
<td>7</td>
<td>4.8 ± 0.020</td>
</tr>
<tr>
<td>lgl4</td>
<td></td>
<td>45-53</td>
<td>204</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>53-68</td>
<td>190</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* fatid dp0 or Fs(2)Ugra, lgd45 dp0 or Fs(2)Ugra, lgf1 Fs(2)Ugra, and, as control, dp0 or Fs(2)Ugra larvae were irradiated by 1000 R of X-rays (250 kV; 0.5 mm Al; 500 R/min).

+ Hours after the initiation of development; 45-53 represents first/early second, 53-68 mid to late second-instar larvae.

Average ± SD.
of the 45–53-hr series and about seven were expected in the 53–68-hr series, no egg-laying females were recovered in the tests of lg1 (< 0.01; χ² test). We conclude that in the absence of lg1 gene function the follicle cells cannot support development of the egg primordia. The lg1 gene therefore has important roles in the follicle cells as well as in the germ line.

**DISCUSSION**

Our results show that five genes required for cell proliferation control in imaginal discs are also required in the female germ line for successful oogenesis and/or subsequent embryogenesis. When the germ line lacks function of the fat, lgd, lgl, doo or c43 genes, the egg primordia either do not develop to the vitellogenic stage, or they develop into abnormal eggs that produce inviable embryos. Mutations in these genes lead to excess cell proliferation in imaginal discs, probably by interfering with cell interactions, and it is possible that they also disrupt cell interactions in the ovary.

lg1 and c43 mutations completely prevented egg production in female germ-line clones. However, a few apparently normal eggs were produced from almost all of the fat clones generated during later stages of larval development. This is probably due to perdurance of the fat⁺ gene product present in the fat/fat⁺ germ line cells at the time of clone induction. Also, more eggs were recovered from the late-induced fat clones than those induced earlier during development. This observation can also be best explained by perdurance of the fat⁺ gene product for the few cell divisions between clone induction and formation of egg primordia (cf: Wieschaus and Szabad 1979). Presumably, the fat⁺ gene product decays or is diluted out in the proliferating fat homozygous cells so that the fat⁺ level eventually becomes subthreshold for egg development. As expected from this interpretation, the few eggs originating from fat homozygous germ line cells were deposited early in the reproductive life of the mosaics.

Embryos did not develop to the stage of cuticle formation in the apparently normal eggs derived from fat homozygous germ line cells, even though these eggs were fertilized by fat⁺-carrying sperm. On the other hand, fat homozygous larvae derived from fat⁺/fat mothers survive until the pupal stage (Bryant et al. 1988). This suggests that maternally-provided fat⁺ substance is normally provided to the embryo and is necessary for embryogenesis. A similar function has been shown for several genes identified by "small disc" and "discless" mutant phenotypes (Perrimon and Mahowald 1986; Taubert and Szabad 1987).

The lgd homozygous germ-line clones were associated with three phenotypes. The more severely affected egg chambers were tumorous, with an excessive number of nurse cells in the degenerating egg primordia, as seen in the ovarian tumor mutants (King and Storto 1988). Thus the lgd mutation, which interferes with cell proliferation control in imaginal discs and other primordia (Bryant and Levinson 1985) appears to also affect cell proliferation control in the developing egg primordium. The second, less severe class was represented by eggs that developed abnormal dorsal appendages. The abnormality of the chorion is perhaps surprising since this structure is made by the follicle cells, which were genetically normal in both the chimeras and the mosaics. Furthermore, our experiments with follicle cell clones indicate that lgd is not required in the follicle cells for normal oogenesis. Therefore, it seems likely that the abnormality results from a failure in communication between the germ line and the enveloping follicle cells, as has been found for a number of other mutations that cause abnormal dorsal appendage formation (Wieschaus, Marsh and Gehring 1978; Schüpbach 1987; Erdelyi and Szabad 1989; Perrimon, Engstroem and Mahowald 1989).

In the third phenotype associated with an lgd germ line, eggs were produced and embryos developed. However, the embryos showed severe head defects, narrow denticle belts and features characteristic of mutations that interfere with dorso-ventral differentiation of the embryo (cf: Konrad et al. 1985; Mayer and Nüsslein-Volhard 1988). Embryos derived from females homozygous for hypomorphic alleles of the maternal-effect gene fs(1)gastrulation defective (Konrad, Goralski and Mahowald 1988) appear almost identical to those produced by lgd and dco, suggesting similar defects in these mutants. Konrad, Goralski and Mahowald (1988) have argued that the fs(1)gastrulation defective gene product is required for cell interactions that are involved in dorso-ventral patterning, and that the mutant phenotype results primarily from failure of ventral furrow formation. The imaginal disc overgrowth mutations might produce a similar maternal-effect phenotype by interfering with the same cell interactions that are affected by fs(1)lgd.

The newly described dominant female-sterile mutation Fs(2)Egtra, which brings about sterility through altered follicle cell functions, made possible the identification of egg primordia with nonmutant germ line and mutant follicle envelope. Our results indicate that homozygosity for fat or lgd mutations in the follicle cells does not prevent egg development. This could mean that these genes are not required for follicle cell functions or, alternatively, that the nonmutant germ line cells can compensate for missing functions of these genes in the follicle cells. A third possibility, that the products of these genes are required in follicle cells but that the mutations behave nonautonomously in
follicle cell clones, seems unlikely in view of the finding (P. BRYANT, unpublished) that both mutations show autonomous development of the mutant phenotype when tested in mosaic imaginal discs. In contrast to fat and lgd, the lgl gene product appears to be necessary for normal function of not only the germ-line but also the follicle cells.

Although the different alleles of dco differ in the severity of their effects on development, they do not form a simple phenotypic series (JURSNICH et al. 1990). dco<sup>18</sup> appears to be more severe than the other alleles in its effect on imaginal discs, and it is also more severe in that it interferes with germ-line development whereas dco<sup>18</sup> does not. However, dco<sup>1088</sup> behaves as a less severe allele than dco<sup>18</sup> when the homologous chromosome carries dco<sup>1088</sup>; dco<sup>1088</sup>/dco<sup>1088</sup> allows more disc development prior to degeneration than is the case with dco<sup>18</sup>/dco<sup>1088</sup> (JURSNICH et al. 1990). Therefore, dco<sup>1088</sup> does not appear to be a null mutation. Nevertheless, since homozygous dco<sup>1088</sup> germ-line cells fail to produce progeny, we conclude that the wild-type allele is required for female germ-line function.

Of all the mutants studied here, only lgd gave rise to tumorous egg chambers. The remainder of the imaginal disc overgrowth mutations do not interfere with egg development by causing additional cell proliferation in either the germ line or the follicle cells. Rather, they cause a simple block in egg development prior to vitellogenesis, or they cause production of abnormal eggs or of eggs which give rise to inviable embryos or larvae. One way of reconciling these different phenotypes with the effects on imaginal discs is to assume that the gene products are necessary for effective cell interaction; cell interaction is required for cell proliferation control in imaginal discs (BRYANT and FRASER 1988), and cell interactions requiring some of the same gene products may be used to control other aspects of development in the ovary. The neoplastic imaginal disc overgrowth mutant dlg, which was previously tested by PERRIMON (1988) and therefore was not included in the present report, does not interfere with oogenesis, but through a maternal function.

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LITERATURE CITED


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