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Genetic requirement of epidermal and female germ line cells in *Drosophila* in the light of clonal analysis

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ABSTRACT Whether the function of a gene is required in a given cell type is often determined through the analysis of clones homozygous for a mutant allele of the gene. The clones usually develop following X-ray induced mitotic recombination. The paper summarizes the conclusions of clonal analyses of different types of mutations in both the epidermis and the female germ line cells of *Drosophila*. Principles of the so called dominat female sterile techmique -for the germ line and the follicle cells- and its use are summarized. Special attention is paid to the genetic requirement of the female germ line due to its fundamental function in the regulation of early embryogenesis.

KEY WORDS: *clonal analysis, mutations, soma and germ line, maternal effect, Drosophila*

Introduction

The traditional and most reliable way of studying whether function of a gene is required in a given cell type is based on the analysis of mosaic spots homozygous or hemizygous for loss-of-function *(m)* mutations. The well-established *Drosophila* genetics and the mosaic-generating systems provide d first the necessary conditions for the analysis of genetic requirements in the imaginal epidermis (mainly in wing and tergite) and subsequently in the female germ line cells. Antonio García-Bellido and his colleagues were among the pioneers of clonal analysis in both the imaginal discs and the female germ line. Their contribution both stimulated the field and made major contributions to our present understanding of phenomena like morphogenesis, perdurance and maternal effects. The present survey summarizes conclusions of the early clonal analysis mainly of the lethal mutations in both epidermis and female germ line cells, and describes the possibility of clonal analysis of the follicle cells.

Principles of clonal analysis

Cells homozygous for a mutation *(m/m)* of interest have been routinely generated during the past forty years through the induction of mitotic recombination in *m/+* heterozygous cells (García-Bellido and Dapena, 1974; García-Bellido and Ripoll, 1978; Fig. 1). For the production of *m/m* cells, *m/+* heterozygous embryos or larvae are irradiated by X- or gamma-rays (Fig. 1). The *m/m* and the +/+ cells may proliferate and give rise to clones in the midst of the *m/+* sibling cells. Marker mutations assist the recognition of the *m/m* and the +/+ cells.

There are two general ways to genetically mark the *m/m* and the +/+ cells. (1) In many cases, recessive marker mutations are linked to both the *m* and the + alleles (Fig. 1A). Following mitotic recombination, one of the daughter cells becomes homozygous for one, the other for the other marker mutation (Fig. 1A). (2) The elimination of a +-linked dominant marker mutation during mitotic recombination is used to the labeling of the *m/m* cells: removal of the dominant mutation leads to the formation of normal cells in the midst of the cells that possess the dominant phenotype (Fig. 1B). Frequency, size, shape and morphology of the *m/m* clones are monitored. Fate of the *m/m* clones tells whether function of the *m*-identified gene is required in the cells or not.

Mitotic recombination can be induced in both the soma and in the germ line by the recently elaborated flip recombination system, a technique discussed by N. Perrimon in this issue (Golic, 1991; Chou and Perrimon, 1996, Perrimon, 1998).

Clonal analysis of the imaginal epidermis

Viable mutations with visible phenotypes. The *m* mutations can be categorized into two classes, depending on whether they allow or not survival of the homozygous individuals. The viable *(m^v)* mutations allow survival of the m^v/m^v zygotes and thus identify non-essential gene functions. Many of these viable mutations are associated with visible phenotypes in the adults. In general, the m^v mutations occur in about 5% of the newly induced mutations. Clonal analysis of the m^v viable mutations clearly classified the mutations into two groups. (1) The majority of the m^v mutations act in cell autonomous manner: the mutant phenotype is observed and is restricted to the m^v/m^v clones. (In fact, several of the m^v mutations are used as cell markers in clonal analyses; García-

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Bellido, 1972; García-Bellido and Dapena, 1974). (2) In a small fraction of the m^v mutations the m^v/m^v clones do not possess the mutant phenotype, indicating that it is either non-autonomous and is compensated by the neighboring heterozygous cells, or the m^v-identified gene function is required in cells other than those included in the m^v/m^v clones.

Zygotic lethal (m^l) mutations. Zygotes homozygous for any of the m^l mutations die before developing to fertile adults. They all identify essential gene functions. In *Drosophila*, about 85% of the newly-induced mutations are zygotic lethals. Moreover, the majority of the female-sterile mutations represent weak (hypomorph) alleles of essential gene functions (Perrimon and Mahowald, 1986; Schüpbach and Wieschaus 1989). Based on the survival of the m^l/m^l clones in the adult epidermis, the m^l lethal mutations can be classified into three categories.

(1) Lethal mutations viable in clones.

Clones of 88-92% of the zygotic lethals survive in the tergites, i.e., clone frequency, size and shape are not affected by the m^l mutation (García-Bellido and Ripoll, 1978; Ripoll and García-Bellido, 1979). Survival of the m^l/m^l clones may be the consequence of one of three reasons: the m^l-identified gene function is (1) not required in the tergite cells, (2) the mutation is not cell autonomous or (3) the $m^l/+$ mother cells provide sufficient amounts of the wild-type gene product to the m^l/m^l daughter cells such that they can survive and function.

(2) Lethal mutations lethals in clones.

Clones of the true cell lethal mutations do not survive, implying that function of the m^l-identified gene is essential for life of the cells. The cell lethal class, however, should be considered with some care. Detectability of clones that contain only a few cells may be problematic. When bristle marker mutations are used, the bristles are usually not included in the small clones. Under such conditions, the small clones are not detected misleadingly suggesting the cell lethal nature of the studied mutation and essential requirement of the m^l-identified gene. However, the small clones appear when single cell marker mutations are used (García-Bellido and Dapena, 1974; Szabad and Bryant, 1982). The discless mutations, that identify essential mitotic functions, are good examples of the lethal mutations that survive only in small clones as was detected in the wings (Szabad and Bryant, 1982). The discless homozygous cells, like all the others that form following mitotic recombination, originate from heterozygous mother cells. The daughter cells share cytoplasm of the mother cell. Products of the mother cell perdure in the cytoplasm of the daughter cells and allow 1-4 divisions of the discless homozygous cells. Virtually all the discless homozygous clones survive on the wing blade, however they are much smaller than their twin partners (Szabad and Bryant, 1982). The phenomenon of persistence of the wild type gene product in the daughter cells and the consequent masking of the mutant phenotype is called “perdurance”, a term coined by García-Bellido and Merriam (1971a). Perdurance is a basic somatic cell phenomenon that essentially corresponds to the maternal effect, a well known phenomenon of embryology. Perdurance plays an especially important role when the clones are induced towards the end of larval life, a few divisions prior to the cessation of cell division in the imaginal disc cells.

It should also be mentioned that, in general, the ml/m^l clones survive much better in tergites (histoblast derivatives) than in wings, eyes or legs (all derivatives of the imaginal discs). The difference is related to the different proliferation dynamics of the two cell types: while the wing disc cells divide in every 8-10 h throughout the larval and the early pupal life (Bryant, 1970; García-Bellido and Merriam, 1971b), the histoblast cells grow in size during larval life and accumulate materials but do not divide. The histoblast cytoplasm is distributed among the daughter cells during a set of cleavage-like divisions within a few hours after the onset of pupariation (García-Bellido and Merriam, 1971c). Accumulation and perdurance of the wild type gene products in the histoblast cells allow normal proliferation, survival and function of the m^l/m^l clones for most of the m^l mutations. In contrast to the tergites, the corresponding m^l/m^l clones die, barely survive or remain small in the wings (Szabad and Bryant, 1982).

(3) Lethal mutations that alter morphology in clones.

Several zygotic lethal mutations have been described that are homozygous viable in clones, however they alter the shape of the clones, indicating that function of the m^l-identified gene is required for the orientation of the mitotic apparatus and/or cell adhesion (García-Bellido and Ripoll, 1978). Clones of other m^l mutations appear normal in size and shape, however some alter hair and/or bristle morphology, others prevent cuticle secretion or coloration (García-Bellido and Dapena, 1974). Several of these latter mutations have been used in clonal analyses. The best examples are *flr*, *pwn*, and *bld* (Lindsley and Zimm, 1992.)

Clonal analysis of the female germ line

The female-sterile mutations. Understanding gene functions in the female germ line has been of special interest in light of the maternal effects, a well know biological phenomenon. The egg cytoplasm contains molecules -besides the yolk nutrients - that ensure coordinated progression of early embryogenesis in both time and space (Davidson, 1976; Wieschaus, 1996). Since there is little if any zygotic gene expression during early embryogenesis not only in *Drosophila* but in all the animal species, the embryos must rely on the maternal dowry for essential functions. Naturally, the maternal dowry is synthesized under the control of the maternal genome, and the components are deposited into the egg during oogenesis. There has been a number of important questions raised that concern maternal effects, such as what types of molecules mediate maternal effects? When and where in the female body are those molecules synthesized? Where and how are mediators of maternal effects deposited in the egg? What are their functions in embryogenesis? What proportion of the genome is involved in the synthesis of the maternal dowry? The most convenient way of answering the above questions is genetic dissection thought the isolation and the analysis of recessive female-sterile *(fs)* mutations that interfere with oo- and/or embryogenesis (Spradling 1993).

About 10% of the newly induced mutations bring about female-sterility. However, the majority of these mutations are weak (hypomorph) alleles of essential loci that, when mutated to the complete loss-of-function (amorph) status, bring about zygotic lethality (Perrimon and Mahowald, 1986; Schüpbach and Wieschaus, 1989). The relatively high frequency of mutations that allow survival of the homozygotes but bring about female sterility suggests higher genetic requirement in the female germ line cells as compared to the soma. The strict *fs* mutations, where the complete loss-of-function (amorph) phenotype is female-sterility, are thus

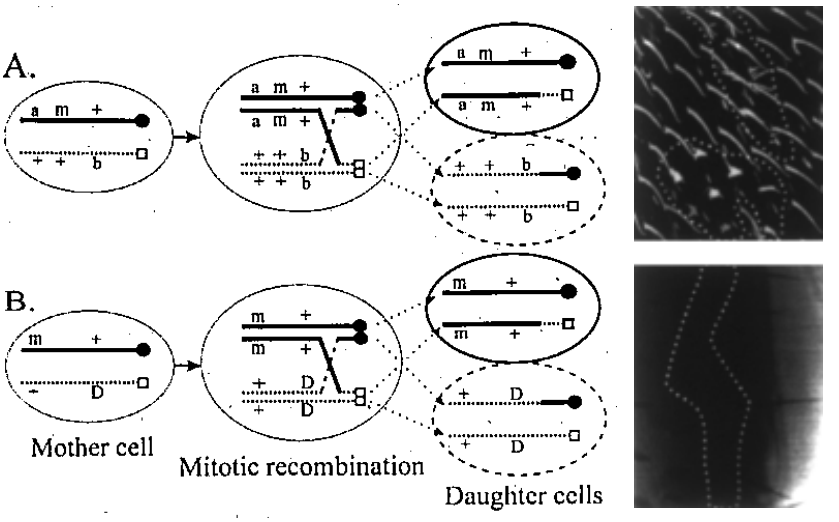

Fig. 1. The principles of the induction of clones through mitotic recombination. (A) *Following mitotic recombination, one of the daughter cells becomes homozygous for the a (and m, the studied mutation) the other for the b recessive marker mutation. A twin spot of a/a and b/b cells form on the wing blade following proliferation and morphogenesis of the wing blade cells. [In the present instance, the a/a and b/b cells are homozygous for the marker mutations multiple wing hairs (mwh) and falre (flr), respectively as encircled on the right panel]. The a/a cells are homozygous for the m mutation. Fate of the a m(a m cells depends on the requirement of the m-identified gene.* **(B)** *The m/m cell does not carry the D dominant marker mutation (that causes e.g., short bristles as shown on the right panel) and hence a clone with normal bristles form. The panel on the right shows a clone of normal bristles on the tergite.*

rare: they comprise only about 1% of the mutations and represent about 100 genes in the *Drosophila* genome (Perrimon and Mahowald, 1986; Schüpbach and Wieschaus, 1989). Obviously, function of much larger than 1% of the genome must be required in the female germ line. Genes with both germ line and zygotic functions are identified by zygotic lethal mutations, a finding that solicited the analysis of the effects of zygotic lethal mutations on the female germ line.

The *fs* mutations are usually classified into two groups (Spradling, 1993). (1) In the "true" *fs* mutations female sterility stems from anatomical and/or functional defects related to ovulation, maturation or deposition of the eggs, and the homozygous females either do not lay eggs or the eggs are abnormal. (2) Females homozygous for any of the so-called "maternal-effect lethal" *(mel)* mutations deposit normal looking and fertilized eggs. However, embryogenesis does not commence or leads to the formation of abnormal embryos that do not hatch from the egg case. The *mel* mutations identify genes that are expressed during oogenesis and their products are required during embryogenesis. Analysis of the *mel* mutations promoted, among others, the understanding of cell differentiation along the anterior-posterior and the dorsal-ventral coordinates of the *Drosophila* embryo (for reviews see Chasan and Anderson, 1993; Driever, 1993; Sprenger and Nüsslein-Volhard, 1993; St. Johnston, 1993).

The female germ line mosaics. Germ line mosaics are usually constructed to elucidate whether functions of the *fs*- or the m^{l}-identified genes are required in the female germ line cells and/or during embryogenesis. There are two procedures to generate germ line mosaics.

(1) Pole cell transplantations and germ-line chimeras

In the course of pole cell transplantations, pole cells -embryonic precursor cells of the future germ line cells- are collected from blastoderm-stage donor embryos and are transplanted into host embryos (Illmensee, 1973; Van Deusen, 1977). The "donor" embryos usually derive from a cross between *fs*/+ or m^{l}/+ heterozygous parents. The "host" embryos derive usually from one of two sources. (1) When their mother is homozygous for a *grandchildless* type of mutation, like $oskar^{301}$ (Lehmann and Nüsslein-Volhard, 1986) or the $topoisomerase\text{-}II^{gs}$ alleles (Erdélyi *et al.,* 1995), pole cells do not develop in the host embryos. (2) Since egg primordia do not develop beyond the early vitellogenic stages, the *Fs(1)K1237*/+ females are also excellent hosts for the "foreign" female germ line cells. *Fs(1)K1237* (also known as ovo^{D1}) is a dominant female-sterile mutation that disrupts function of the germ line cells (Komitopoulou *et al.,* 1983; Perrimon, 1984).

Following pole cell transplantations, several of the host embryos develop to adult germ line chimeras with normal soma and mutant germ line. It may be concluded, when the germ line chimeras show the mutant phenotype, that function of the *fs*- or the m^{l}-identified gene is required in the germ line. Absence of the mutant phenotype in the germ line chimeras suggests somatic gene requirement.

The question whether the so-called soma-dependent *fs* mutations affect function of the somatic cells of the gonads or other organs is usually determined through the analysis of ovarian chimeras. Two types of chimeras are generated by transplantation of larval ovaries: (1) *fs/fs* ovaries are implanted into usually *Fs(1)K1237/+* host larvae or (2) genetically marked ovaries are implanted into the *fs/fs* larvae, and the *fs*-associated mutant phenotype is subsequently analyzed in the adult ovarian chimeras.

Analysis of germ-line chimeras showed, e.g., that mutations that interfere with sex determination have no effect on the germ line (Schüpbach, 1982), that germ line and soma cooperate during egg development in *Drosophila* (Schüpbach, 1987) and that most of the *mel* mutations that alter embryonic body pattern are germ-line dependent (Schüpbach and Wieschaus, 1986). In fact, analyses of germ line chimeras were of immense help in the elaboration of genetic hierarchy required to establish both the dorsal-ventral and the terminal axis determination systems during *Drosophila* embryogenesis (Chasan and Anderson, 1993; Sprenger and Nüsslein-Volhard, 1993).

Germ-line chimeras were also generated for a number of m^{l} mutations (Taubert and Szabad, 1987; Szabad *et al.,* 1991). Effects of the m^{l} mutations on the germ line will be discussed together with results of the germ line clonal analysis.

(2) Germ line clones from mitotic recombination

(A) The "classical" recessive marker mutation method. Cell-lineage relationships of the *Drosophila* female germ line were

elucidated through the analysis of germ line clones that originated following the induction of mitotic recombination: one of the germ line cells was made homozygous for *fs(1)K10*, the other for *maroonlike*, two germ-line dependent recessive marker mutations (Wieschaus and Szabad, 1979). Since the female germ line (oogonial) cells are diploid and proliferate mitotically throughout the larval and early pupal life (King, 1970), they are ideal targets for the induction of mitotic recombination and clonal analysis.

To study whether a set of *fs* mutations alters the function of the germ line cells, i.e., whether the function of the *fs*-identified gene is required or not in the germ line cells, Wieschaus *et al.* (1981) used the arrangement shown on Figure 1A, in which -following mitotic recombination- one of the daughter cells becomes homozygous for both fs(1)K10 and the studied *fs* allele. In two of the early oogenesis defective and in four of the fragile-collapsed egg *fs* mutations the mutant phenotype developed in the germ line clones, showing that functions of the *fs*-identified genes are required in the germ line. Two egg retention, a short egg and one fragile-collapsed egg *fs* mutations had no effect in the germ line clones indicating that functions of the corresponding genes are not required in the germ line but rather in either the ovarian or some other types of the soma.

(B) The dominant female-sterile technique. The Wieschaus, Audit and Masson (1981) paper is, to my best knowledge, the only one where a recessive marker mutation was used for the labeling of female germ line cells homozygous for *fs* mutations. From the early eighties on, the female germ line clones were produced according to the arrangement shown in Figure 1B using dominant female sterile *(Fs)* mutations for the induction of germ line clones: larvae trans-heterozygous for the *fs* (or an m^l) and an *Fs* mutation are irradiated for the induction of mitotic recombination. One of the daughter cells becomes homozygous for the *fs* (or the m^l) mutation and looses the *Fs* mutation and its detrimental effect. The procedure is called the "dominant female-sterile technique" (Wieschaus, 1980; Perrimon and Gans, 1983). Fate of the *fs/fs* (m^l/m^l) cells reveals whether function of the *fs*- or m^l-identified gene is required in the female germ line and/or during embryogenesis: the gene function is required whenever the mutant phenotype develops in the germ line clones and is non-essential when the studied mutation has no effects on the germ line clones.

The *Fs* mutations used for the germ line clonal analyses need to alter function of the germ-line cells (should be germ-line dependent) and prevent egg deposition. Originally there were three such *Fs* mutations available: *Fs(2)D* on the right arm of the second chromosome (Yarger and King, 1971; Wieschaus 1980), *Fs(1)K1237* (=ovo^{D1}) on the first (X) chromosome (Komitopoulou *et al.,* 1983; Perrimon, 1984) and *Fs(2)1* on the left arm of the second chromosome (Szabad *et al.,* 1987,1989). All three have been extensively used for the analysis of germ line clones. More recently Perrimon and coworkers have introduced ovo^{D1} containing transgene on all chromosome arms expanding the dominant female sterile technique to all major chromosome arms (Chou and Perrimon, 1996; Perrimon, 1998).

Several mutations have already been analyzed by the dominant female sterile technique. It is apparent that function of the sex determination genes is not required in the female germ line of *Drosophila* (Schüpbach, 1982). Function of about 50% of the *fs* mutations affect germ line functions, the other 50% do not (Perrimon and Gans, 1983; Spradling, 1993). Germ line clonal analysis of the m^l zygotic lethal mutations are of outstanding importance and will be covered in the following paragraph.

The effects of zygotic lethal mutations on the female germ line in Drosophila

Several laboratories have made use of the dominant female sterile technique and induced germ line clones of m^l mutations (García-Bellido and Robbins, 1983; Perrimon *et al.,* 1984; Perrimon and Mahowald, 1986; Wieschaus and Noell, 1986; Taubert and Szabad, 1987; Szabad *et al.,* 1991, Perrimon *et al.,* 1996; see also Perrimon, 1998). Based on their effects on the female germ line cells, the m^l mutations can be classified into three types.

(1) Zygotic lethal mutations viable in female germ line clones.

About 27-33% of the m^l mutations have no effects on neither the female germ line cells nor on the embryos that develop in eggs of germ line clone origin (García-Bellido and Robbins, 1983; Perrimon *et al.,* 1984). This class includes, among others, the lethal *bithorax complex* mutations (Kerridge and Dura, 1982). It is apparent that function of the m^l-identified genes in this class is not required in the female germ line cells or the mutant phenotype is not cell autonomous. However, function of the other 67-73% of the zygotic lethal mutations do affect female germ line function. The 67-73% is in great contrast to the 8-12% reported for the tergites (García-Bellido and Ripoll, 1978; Ripoll and García-Bellido, 1979), and indicate immense genetic requirement in the female germ line cells (García-Bellido and Moscoso del Prado, 1979).

(2) Zygotic lethal mutations that are lethal in female germ line clones.

About 30% of the zygotic lethal mutations are not viable in germ line clones indicating essential gene function of the m^l-identified genes in the female germ line cells. [Perdurance of the wild type gene product from the heterozygous mother cell may allow development of a few eggs from the m^l/m^l clones (Perrimon *et al.,* 1984, Perrimon and Mahowald, 1986). The disturbing effect of perdurance may especially be significant when the clones are induced towards the end of larval life. Perdurance looses its importance along aging of the females carrying the m^l/m^l germ line clones, i.e., the wild type product becomes diluted and/or decays. Dilution and/or decay of the perduring wild type product.]

It is rather curious that zygotes homozygous for the germ line lethal mutations can develop to embryos, larvae and even pupae. The phenomenon indicates that products of the m^l-identified genes are provided in the eggs of the $m^l/+$ mothers as part of the maternal dowry, and the maternally provided egg components support development of the m^l/m^l zygotes (Szabad and Bryant, 1982; Mahowald *et al.,* 1984; Perrimon and Mahowald, 1986; Taubert and Szabad, 1987).

(3) Zygotic lethal mutations with maternal effect phenotypes.

The homozygous condition for about 40% of the zygotic lethal mutations is compatible with germ cell viability and eggs may derive from the m^l/m^l female germ line clones. However, since the cytoplasm of these eggs does not contain the wild type gene product to support embryogenesis, abnormal embryos develop in the eggs (Perrimon and Mahowald, 1986; Perrimon *et al.,* 1996). The phenotype of these embryos is very different from that of the

m^l/m^l zygotes that derive from $m^l/+$ parents. The difference between the two phenotypes stems from the presence of the wild type products in the eggs of the $m^l/+$ females and clearly reflects a maternal effect of the m^l-identified gene.

It has been well established that the maternally-provided wild type gene product masks the "real" mutant phenotype in a number of zygotic lethal mutations and hence essential functions of the m^l-identified gene go undetected. Of several examples for the above phenomenon *lethal(1)polehole [l(1)ph]* is probably the best known (Perrimon, 1984; Perrimon *et al.,* 1985). The *l(1)ph/Y* larvae of the *l(1)ph/+* mothers and the +/Y males die at the end of larval life due to defective proliferation of the imaginal disc cells. In contrary, the *l(1)ph/Y* zygotes that derive from *l(1)ph/l(1)ph* female germ line cells die as embryos and possess typical *torso* mutant phenotype, showing that the wild type *polehole*$^+$ gene is a member of the so-called terminal group genes (Perrimon *et al.,* 1985; Sprenger and Nüsslein-Volhard, 1993). The fate of the embryos that derive form *l(1)ph/l(1)ph* female germ line cells depends on the sperm-derived allele: typical *torso* embryos develop if the sperm does not contribute an *l(1)ph*$^+$ allele. In case of "paternal rescue", however, the sperm-derived wild type allele can compensate the lack of the maternal contribution and larval development proceeds (Perrimon *et al.,* 1985). Since a maternal effect of lethal mutations is a rather common phenomenon in *Drosophila*, germ line clonal analysis has almost become a routine.

Taking together the effects of the *fs*, *mel* and the m^l mutations on the female germ line, it is apparent that function of about 30% of the *Drosophila* genome is required for viability of the female germ line cells. Germ line clonal analysis of the *mel* and the m^l mutations clearly showed that at least 70% of the *Drosophila* genome is required during oogenesis and the products are present in the egg cytoplasm (the maternal dowry) to support embryogenesis (García-Bellido and Moscoso Del Prado, 1979; García-Bellido and Robbins, 1983).

Clonal analysis of the follicle cells

It has long been known that germ line and somatic components of both the gonads and the body act in harmony during egg development (King, 1970; Schüpbach, 1987; Anderson, 1995; González-Reyes *et al.,* 1995). The analysis of *fs* and *mel* mutations have already revealed essential gene functions in the follicle cells for egg and/or embryonic development. However, the understanding of the genetic requirement of the follicle cells awaits for evaluation. It may be expected that follicle cell clones of some zygotic lethal mutations will alter oogenesis and/or egg development. There may exist genes -identified by m^l mutations- that are expressed in the follicle cells, their products are maternally provided and are required for embryonic pattern formation. These possibilities can be studied in follicle cell clones of the zygotic lethal mutations.

The diploid follicle cells are derivatives of the gonadal mesoderm and increase in numbers by mitosis throughout larval development (Szabad and Hoffmann, 1989; Cumberledge *et al.,* 1992; Szabad and Nöthiger, 1992). Follicle cells of the egg primordia originate from a lining of follicle stem cells in the germaria of the ovarioles (King 1970). Mitotic recombination can thus be induced in the follicle primordial cells providing the possibility for the formation of *fs* or m^l homozygous follicle cell clones (Wieschaus *et al.,* 1981; Szabad *et al.,* 1991).

The *Fs* mutations *Ugra* and *Apc* (on the left arm of the second and on the left arm of the third chromosomes, respectively) are excellent tools to extend the dominant female sterile technique to the follicle cells (Szabad and Hoffmann 1989; Szabad *et al.,* 1991). Both *Ugra* and *Apc* disrupt function of the follicle cells without altering germ line functions (Erdélyi and Szabad 1989; Szabad *et al.,* 1989). The *Ugra/+* and the *Apc/+* females do not lay eggs (or lay only very few fragile-collapsing eggs) and hence meet the requirements to be used as tools for the dominant female sterile technique. In fact, *Ugra* has already been used to elucidate the effects of *l(2)fat, l(2)giant discs* and *l(2)giant larvae*, three of the *disc overgrowth* zygotic lethal mutations, in follicle cell clones (Szabad *et al.,* 1991). *Ugra/fat (gd or gl)* larvae were irradiated for the induction of *fat/fat (gd/gd* or *gl/gl)* follicle cell clones and egg production of the developing females was monitored. The results clearly showed that while function of the *fat*$^+$ and the *gd*$^+$ genes is not required in the follicle cells (i.e., eggs develop from the mutant homozygous clones with the control rate and adults derive from the eggs), function of the *gd*$^+$ gene is indispensable in the follicle cells.

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