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Maternal effect embryonic lethal mutants identified in the $37D_2$–$38A_1$ region of chromosome 2 of *Drosophila melanogaster*

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Abstract. The genome region represented by *Deficiency* (*2L*) *E55* in *Drosophila melanogaster* has been implicated in maternal gene activity, based on high levels of F_1 embryonic mortality in hemizygous *Df* (*2L*) *E55*/+ female × +/+ male matings. Extensive mutagenesis screens were conducted to detect any maternal embryonic lethal loci in the *Df* (*2L*) *E55*, $37D_2$–$38A_1$ cytological region. Consequently, seven maternal effect embryonic lethal loci have been identified. Five of them altered the ventral embryonic cuticle pattern in various ways. In addition to these, a new ovarian tumour mutant has also been identified in the same region. This demonstrates that maternal haplo insufficiency of the *Df* (*2L*) *E55*/+ females, leading to embryonic mortality, is due to the *en bloc* removal of the wild type alleles of these mutants, whose activity is required in the female for ovarian organisation and embryonic development.

Keywords. *Drosophila melanogaster*; *Df*(*2L*)*E55*; maternal effect embryonic lethal.

1. Introduction

In the development of higher organisms, an apparently homogenous egg cell gives rise to a complex but strictly organised embryo and later to an adult. The structural organisation of the *Drosophila* embryo is under the control of several maternal and zygotic genes (Lewis 1978; Nusslein-Volhard and Wieschaus 1980; Wieschaus *et al.* 1984; Schupbach and Wieschaus 1989). The basic spatial organisation of the embryo with respect to its anterior–posterior and dorsal–ventral surface is defined before fertilization and requires maternal gene activity (Bull 1966; Nusslein-Volhard 1979; Lohs-Schardin 1982). After fertilization, more specialised aspects of embryo structure, such as segment number, position and identity, are programmed by zygotic genes (Struhl 1981, 1982). It has been shown that the maternal information in the egg leads to position-specific responses of the blastoderm nuclei such that cell fate is stably set (Lewis 1963, 1978; Wright 1970; Garcia-Bellido 1975; Ingham 1988).

The nature and distribution of this maternal information is being gradually revealed with the help of classical genetic, biochemical and molecular cloning techniques. An indispensable method which forms the basis for such analyses is the discovery of new maternal effect mutant genes (Rice 1973; Bakken 1973; Gans *et al.* 1975; Mohler 1977; Schupbach and Wieschaus 1989).

A different approach to evaluate the maternal genomic component is to study the effect of deletions on embryonic development. In one such study, Garcia-Bellido *et al.* (1983) identified 10 deletions on chromosomes 2 and 3 which were maternally haplo-insufficient for normal embryogenesis. This indicated that these deletions probably uncovered maternally acting genes. Some of these maternally haplo-insufficient regions *Df* (*2L*) *165*, *Df* (*2L*) *137* and *Df* (*2R*) Vg^B uncover known

maternal effect genes (Nusslein-Volhard 1977, 1979; Schupbach and Wieschaus 1986, 1989). However, none of them have been genetically analysed in detail to study if they actually uncover any maternally acting genes.

In the present study, large scale mutational analysis of one such region uncovered by *Df (2L) E55* has been attempted to identify any possible *maternal effect embryonic lethal* (*mel*) mutants. This is a medium-sized deletion of 15 polytene bands ($37D_2$–$38A_1$) and uncovers three known loci–*ref* (2) *P*, *pads* and *spitz*, none of which are maternal mutants. *ref* (2) *P* is a CO_2-sensitive virus-resistant mutant, *pads* is a wing morphology mutant and *spitz* a zygotic lethal.

As a result of this saturation screening of the *Df (2L) E55* region, seven *mel* mutant genes, one *ovarian tumour* mutant and two alleles of the *pads* locus were identified. The *mel* mutants have been studied for their ventral cuticular phenotypes and some for their embryogenesis. The results of these observations and the conclusions drawn from them are presented in this paper.

2. Materials and methods

2.1 *Growth medium*

Flies were reared on standard fly food made of cornflour, unrefined sugar and agar, supplemented with dry yeast granules.

Eggs were collected on a synthetic orange-flavoured agar medium containing 15 g agar, 20 g yeast and 50 ml prepared synthetic orange drink (Rasna) per litre of water.

2.2 *Stocks*

For mutagenesis, a freshly isolated lethal-free *dp b cn* marked second chromosome was used. The *CyO* (In 2LR) *O* $dp^{lvI}Cy$ *pr* cn^2 $DTSL^{513}$ is a second chromosome balancer bearing a dominant temperature sensitive lethal (*DTSL*), viable when raised at 19°C and lethal at 28°C. This was obtained from the Bowling Green Stock Centre, USA. The *Df (2L) E55* bearing chromosome was marked with *rdo hk pr* and was obtained from the Pasadena Stock Centre, California, USA.

The description of marker mutants and deficiencies may be found in Lindsley and Grell (1968) and Lindsley and Zimm (1985, 1987).

2.3 *Mutagenesis and establishment of female sterile lines*

Mutagenesis of 2–3 day old *dp b cn* males was carried out by feeding 6 h prestarved flies for 6 h on a solution of 0·03 M EMS in 2% sucrose. The treated males were mated to *CyO/Df (2L) E55 rdo hk pr* virgin females in a 1:3 ratio. The F_1 males of the genotype *dp b cn*/Df (2L) E55 rdo hk pr* were singly mated to 2–3 *CyO* $DTSL^{513}$*/Df (2L) E55* virgin females in vials. They were maintained at 19°C for 5–6 days after which the parents were transferred into fresh vials and kept at 28°C for another 5–6 days and then discarded. These two sister vials were given identical

serial numbers. The F_2 flies which eclosed at 28°C were entirely of the genotype *dp b cn*/Df (2L) E55* since the other categories of flies were eliminated either due to the DTSL or the homozygosity of the deficiency (figure 1). The surviving flies from each vial represented the progeny of independent EMS treated sperms, and were tested for the induction of a mutation in the region uncovered by the deficiency. Flies from each vial were individually tested for female sterility by a modified block agar method (Wieschaus and Nusslein-Volhard 1986). The sterile lines thus identified were retrieved as heterozygotes from their sister vials at 19°C. The *CyO* (*In 2LR*) O *dp^lvI Cy pr cn*^2DTSL^513/*dp b cn** flies had orange eye colour due to the *cn* mutant in the homozygous condition, while *CyO DTSL*^513/*Df (2L) E55 rdo hk pr* flies had purple eye colour.

Maternal effect lethality of the sterile lines was subsequently confirmed (see § 3.1) after multiplying the mutant lines.

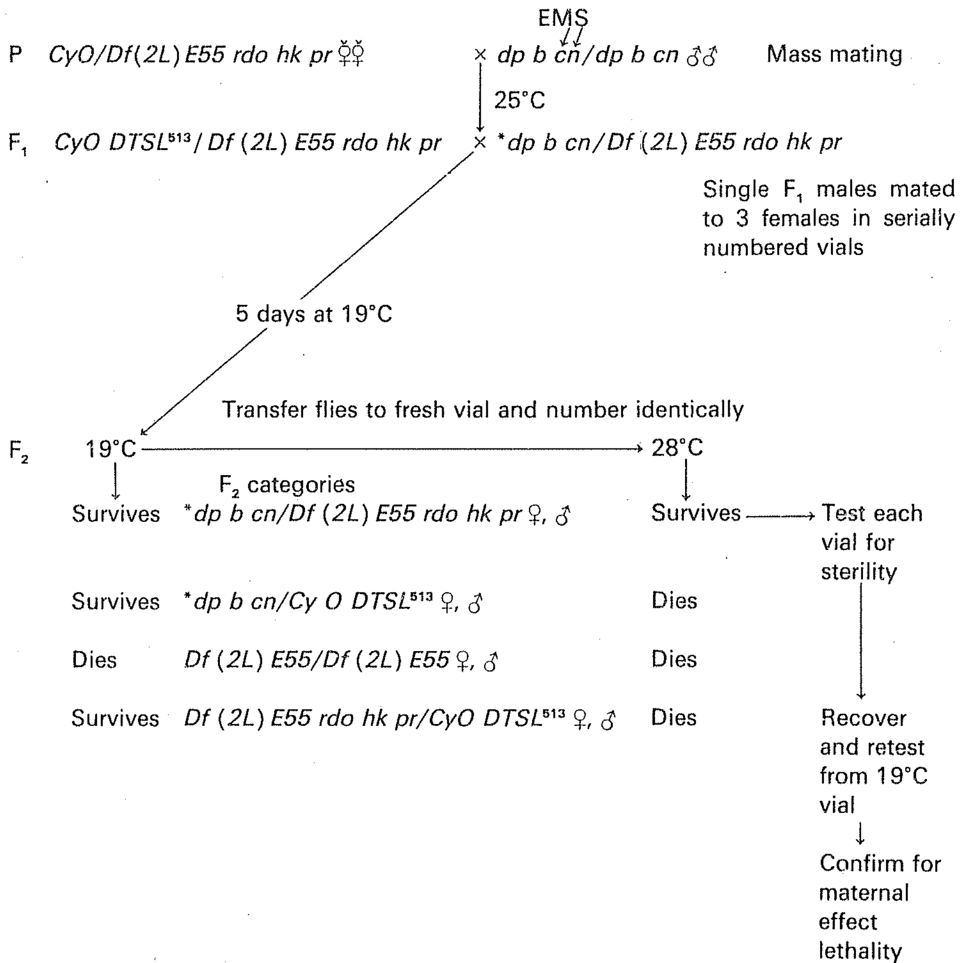

Figure 1. Scheme for generation of mutant hemizygous lines for screening of maternal effect lethals in the $37D_2$–$38A_1$ region.

2.4 *Screening embryos*

Eggs were collected in large numbers from the maternal effect lethal line by the split-bottle method (Lawrence and Johnston 1986) and cleared in Hoyer's medium for whole mount preparation (Van der Meer 1977). Live embryogenesis was microscopically observed in bright field illumination, by immersing clean eggs in mineral oil under coverslips supported by thin wire rodlets.

Photographs were taken on a Nikon optiphot FX photomicroscope.

2.5 *Complementation*

Crosses were made between mutants, and eggs were collected from 10–15 females (*trans* heterozygous for two *mel* mutants) mated to wild-type males, using the block agar method. Unhatched eggs were inspected for mutant phenotypes after clearing in Hoyer's medium.

3. Results

3.1 *Mutant screening*

The crossing scheme for the establishment of mutants in the *E55* region of the *dp b cn* chromosome is illustrated in figure 1. This scheme makes use of the following:

(a) The elimination of deficiency-homozygous and $DTSL^{513}$-bearing flies at 28°C to generate large numbers of *dp b cn*/Df (2L) E55* flies from each cross. This obviates the necessity of etherizing and separating the required class of flies from each vial.
(b) The uncovering of any mutation in the $37D_2$–$38A_1$ region of the *dp b cn* chromosome by the deficiency.
(c) The elimination of male sterile and zygotic lethals in the *E55* region.

A total of 10,000 F_1 crosses were made, of which 6,325 yielded sufficient F_2 progeny at 28°C. Flies from each of these vials, which were all of the genotype *dp b cn*/Df (2L) E55* were allowed to lay eggs and the frequency of hatching was observed. Putative mutants were isolated on the basis of 100% embryonic mortality at this step which was repeated once or twice with flies from the F_2 vial. To confirm the occurrence of a maternal effect mutation, *dp b cn*/Df (2L) E55* virgin females from the sister vials at 19°C were collected and mated to *Canton-S* males and their embryos observed for their ability to hatch. When this cross yielded 100% embryonic lethality the *dp bn cn*/CyO* DTS^{513} flies were recovered and the mutant line purified and retested after multiplication for the elimination of female steriles from the maternal mutant class.

Of the 6,325 F_2 lines tested, 11 recessive and 2 dominant maternal effect embryonic lethal lines were identified. Several of these mutants had *cis* associated lethals which were outcrossed before performing further investigations. All the embryonic lethal phenotypes were found to be independent of the paternal genotype.

The 13 mutants fell into 7 complementation groups which are numbered *mel 1* to *mel 5*, *Mel 6* and *Mel 7* (table 1). The *mel 1*, *Mel 6* and *Mel 7* loci had one allele

Table 1. List of *mel* complementation groups and their phenotypes.

Complementation group	No. of alleles	External egg structure	Embryonic phenotype at the stage of arrest
mel 1	1	Short egg, stubby chorionic appendages	32–64 cleavage nuclei
mel 2	3	Normal	*mel* 2^3–syncitial blastoderm *mel* 2^1, *mel* 2^2–cephalolaryngeal skeleton deleted
mel 3	3	Short egg, transluscent chorion, fused and stubby chorionic appendages	*mel* 3^1–8–16 nuclei *mel* 3^2, *mel* 3^3–posterior abdominal segments deleted
mel 4	2	Normal	Cephalo-laryngeal skeleton absent, abdominal segments narrow in width
mel 5	2	Normal	Variable between syncitial and cellular blastoderm
Mel 6	1	Normal	Club-shaped head, weakly differentiated abdominal segments
Mel 7	1	Normal	Twisted embryonic cuticle, reduced ventral cuticle bands

each, *mel 4* and *mel 5* had 2 alleles, and *mel 2* and *mel 3* had 3 alleles each. In addition to these, one recessive agametic line was also recovered in the *E55* region, that was later observed to be due to the formation of ovarian tumours. This is designated as a new *ovarian tumour* (*ovt*) locus in this region. Two alleles of *pads* were also recovered in this screen.

The 7 *mel* loci identified in this experiment were found necessary for normal embryonic development as evidenced by abnormalities seen during embryogenesis and in the ventral culticular patterns of the dead, differentiated embryos. Presented below is a brief description of the developmental abnormalities observed in the 7 *mel* loci.

3.2 *Embryonic phenotypes of the maternal effect lethals*

3.2a *mel 1*: Embryos of *mel 1* females were arrested after 4–5 zygotic cleavage divisions. The stained cleavage nuclei appeared as dense sticky chromatin clumps a few hours after arrest. Externally the eggs were wider in diameter and shorter in length and had short and stubby chorionic appendages.

3.2b *mel 2*: Three alleles were recovered at this locus. The embryos derived from *mel* 2^1 and *mel* 2^2 females completed embryonic development but showed abnormal cuticular differentiation. In *mel* 2^3 the embryos were arrested at the syncitial blastoderm stage, when 4-500 nuclei could be seen (figure 2). No cellularization was observed in these embryos.

Cuticular phenotype: In *mel* 2^1 embryos the cephalo laryngeal skeleton was entirely deleted (figure 3b). The chitinous denticle bands of the abdominal segments were also reduced. The *mel* 2^2 allele had a milder phenotype where the dorsal pharyngeal arms, maxillary hooks and ventral arm of the head skeleton were

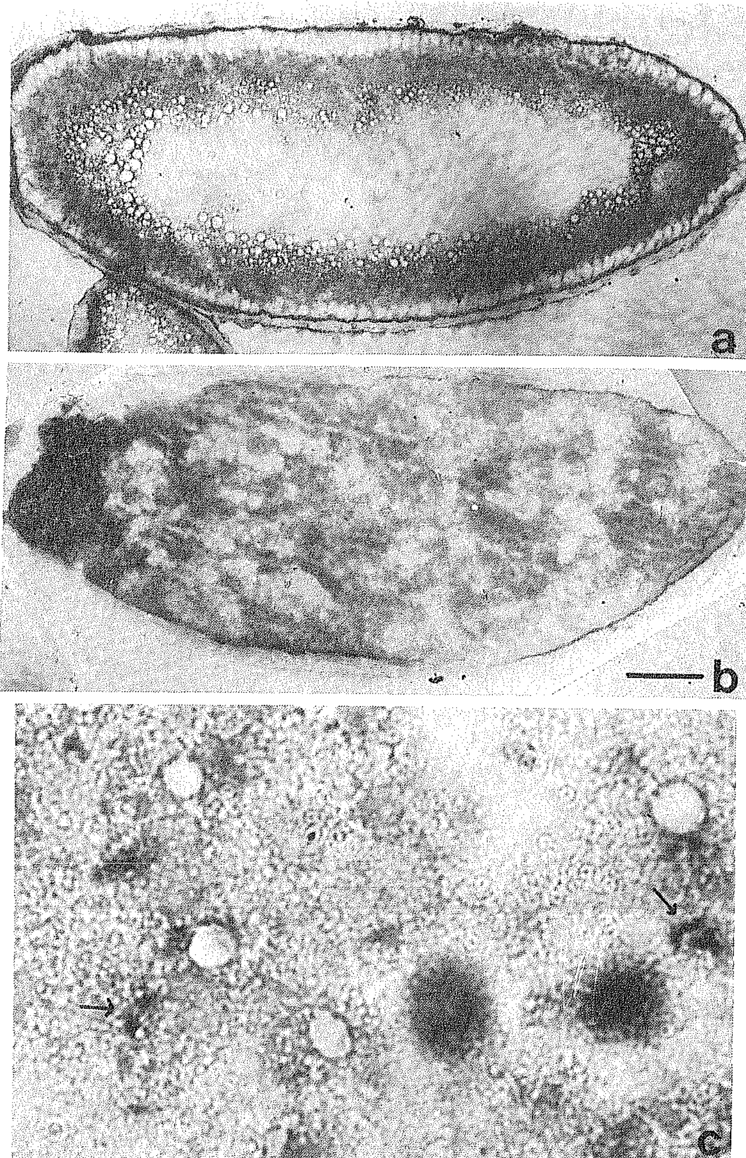

Figure 2. Tissue sections of embryos (**a**) derived from a wild-type female (age of embryo: 2·5 h), (**b**) derived from a *mel* 2^3/*mel* 2^3 female (age of embryo: 24 h); note the absence of nuclear migration (bar equals 50 μm). (**c**) squash preparation (aceto-orcein stained) of an embryo from a *mel* 2^3 female showing dividing nuclei (→)

partially developed (figure 4b) with an undifferentiated chitinous mass near the median tooth.

Embryogenesis: This was studied in *mel* 2^1, where the earliest visible deviation from normal development occurred during head involution (11 hours after egg laying). In wild-type embryos the head involutes to form a slanting convex frontal

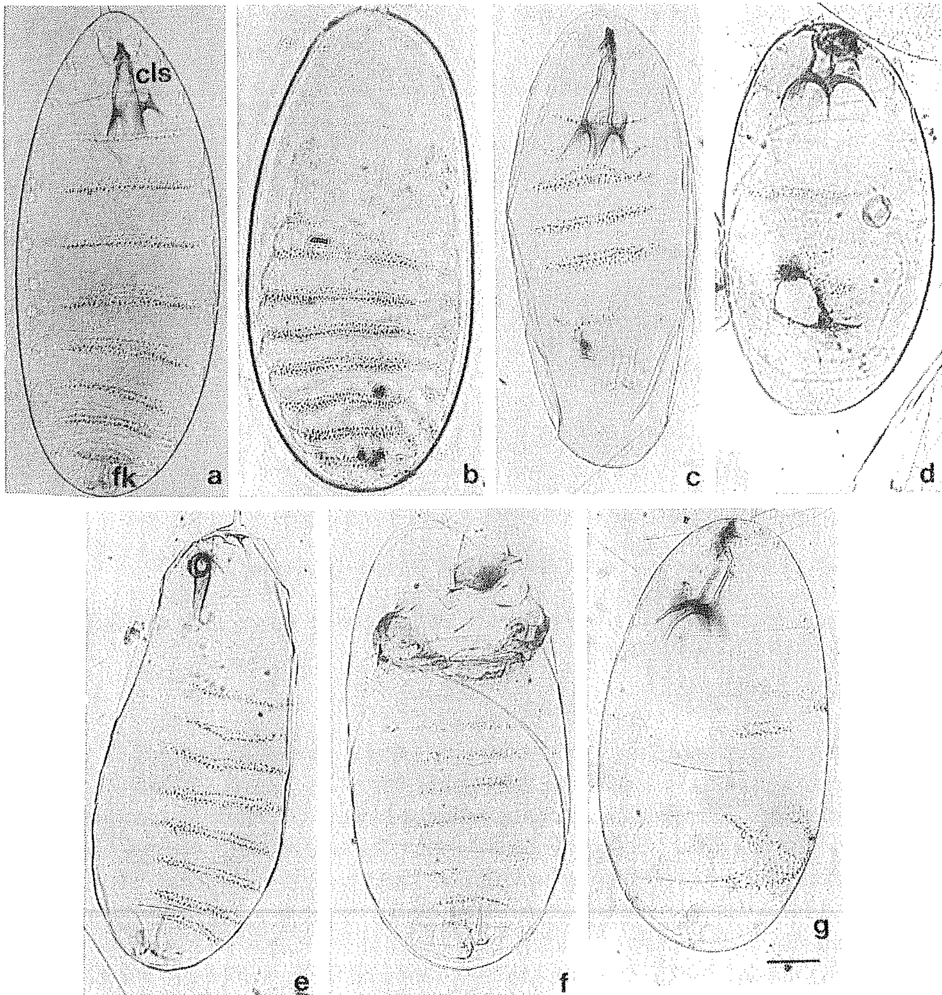

Figure 3. Ventral cuticular patterns of embryos derived from (**a**) wild type, (**b**) *mel* 2^1/*mel* 2^1, (**c**) *mel* 3^2/*mel* 3^2, (**d**) *mel* 3^3/*mel* 3^3, (**e**) *mel* 4^1/*mel* 4^1, (**f**) *Mel 6/Df(2L)E55*, (**g**) *Mel 7/ Df(2L)E55* females. (Bar represents 50 μm.) Abbreviations: Cls-Cephalolaryngeal skeleton, fk-Filzkorper.

sac across the anterior tip of the embryo (figure 5e). In mutant embryos, the frontal sac was very reduced at the anterior ventral tip (figure 5j).

In *trans* heterozygotes the mel 2^3 phenotype was recessive to the mel 2^1 and mel 2^2 phenotypes.

3.2c *mel 3*: Three alleles at this locus were identified. Their common feature was short egg length, translucent chorion and stubby fused chorionic appendages. All the embryos of *mel* 3^1 females developed to form an early syncitial blastoderm of 8–16 nuclei but stopped development subsequently. In the *mel* 3^2 and 3^3 alleles, fully differentiated embryos were produced, but with distinct cuticle pattern abnormalities.

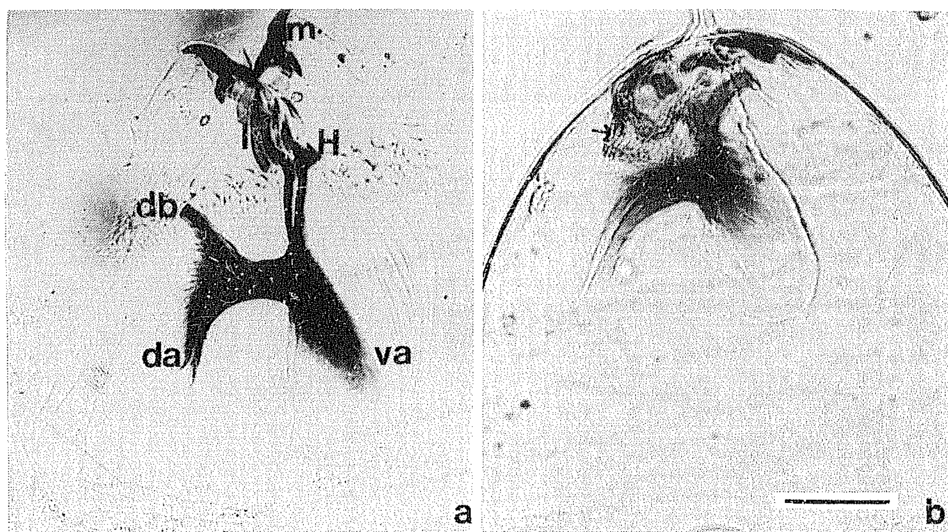

Figure 4. Head stuctures of larvae derived from (**a**) wild type, (**b**) *mel* 2^2/*mel* 2^2 females. Note chitinous proliferation in (b).
Abbreviations: m = mouth hooks, l = labrum, da = dorsal arm, va = ventral arm, db = dorsal bridge, H = H piece. (Bar represents 25 μm.)

Cuticular phenotype: The most characteristic feature of *mel* 3^2 and *mel* 3^3 was the deletion of the posterior abdominal segments. Embryos of *mel* 3^3 had A_2/A_3–A_8 segments deleted. The only segments observed were A_1 and occasionally A_2 (figure 3d). The cuticular region posterior to A_1/A_2 had a large ventral hole with traces of denticles around its circumference. There was no evidence of posterior spiracle and filzkorper development. In these embryos the head structures were also reduced and abnormal.

In *mel* 3^2, the abdominal deletions were milder and were reduced to the posterior 3–4 segments. The abdominal segments A_5/A_6–A_8 were either entirely or partially deleted and fused with each other. The spiracles and filzkorper were normally formed and so were the head structures (figure 3c).

Embryogenesis: The earliest visible abnormality in *mel* 3^2 and *mel* 3^3 embryos was the absence of pole cells (figure 5f and g). Also the dorsally flattened polar plate seen in wild-type embryos (figure 5c) was never observed in the mutants. Later the posterior mid-gut furrow, instead of proceeding anterodorsally, plunged ventrally in the mutants (figure 5h). The cephalic furrow too was weak and delayed in *mel* 3^3.

In *trans* heterozygotes the mel 3^3 phenotype was dominant over the mel 3^2 and mel 3^1 phenotypes. Their allelic relationship was mel 3^3 > mel 3^2 > mel 3^1. The 3^3 and 3^2 alleles in *trans* over 3^1 or the deficiency had more extreme phenotypes while the 3^1 allele over the *Df* (*2L*) *E55* was indistinguishable from the homozygote.

3.2d *mel 4*: Two alleles *mel* 4^1 and *mel* 4^2 were identified in this screen.

Cuticular phenotype: The chitinous head skeleton was completely deleted in embryos derived from mutant females (figure 3e), with only traces of the mouth

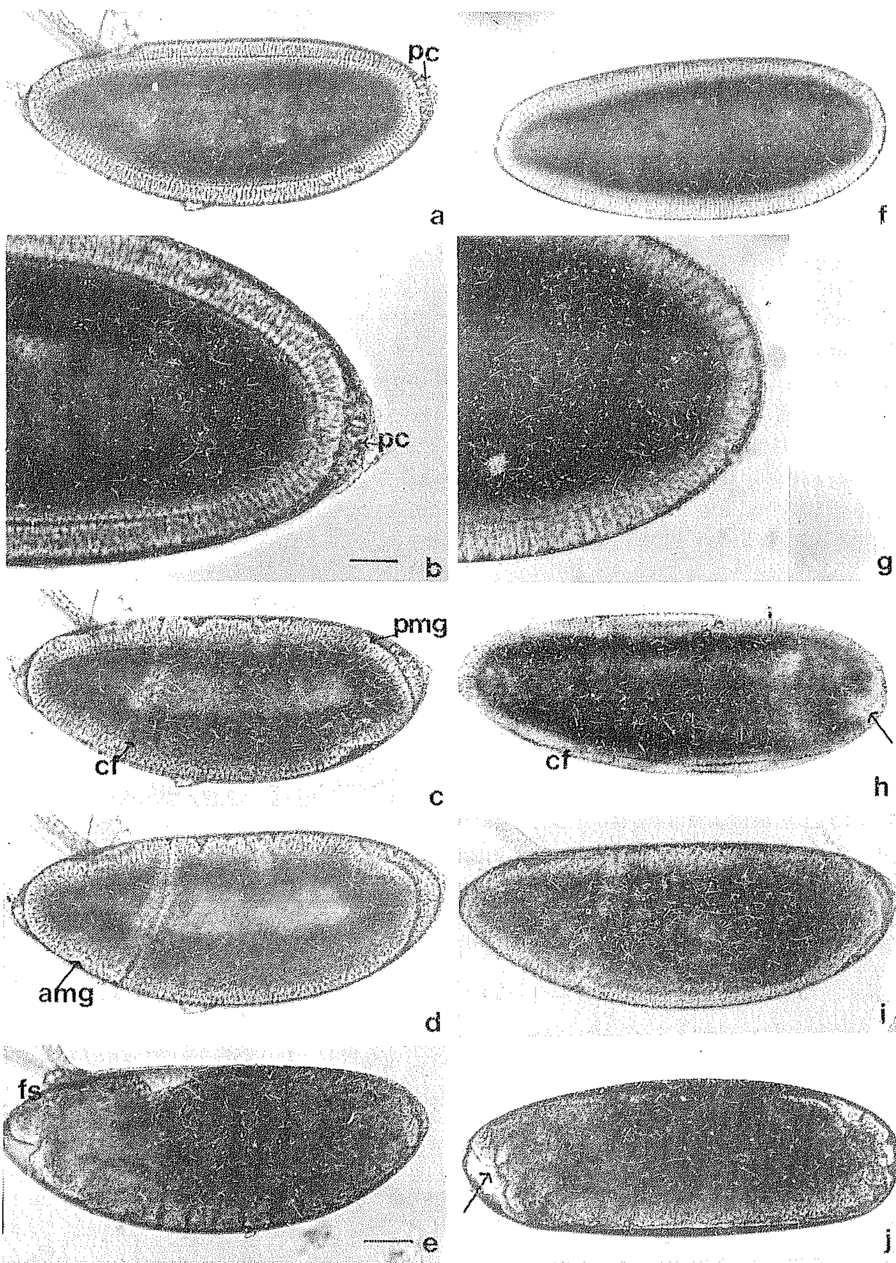

Figure 5. Gastrulation in embryos of wild-type (a)–(e) and those derived from various *mel* females (f)–(j). Pole cells (PC) present in wild-type (a) and (b), and absent in embryos of *mel* 3^3 females (f) and (g). Posterior mid-gut furrow (PMG) occurring at its normal dorsal position in wild-type. (c). Also note normal cephalic furrow (CF) in the same embryo. Abnormal ventrally plunging PMG (→) and weak CF in embryos of *mel* 3^3 females (h). Anterior mid-gut furrow (AMG) present in wild-type (d) and absent in embryos of *mel 4* females (i). Normal frontal sac (FS) formed in wild-type embryos (e). Reduced FS in embryos of *mel* 2^1 females (j), (Bar in b = 50 μm and in e = 100 μm.)

hooks. In several cases there was an open anterior hole. The denticle bands of the thoracic segments were differentiated poorly and the abdominal denticles were narrower in width than normal.

Embryogenesis: The anterior mid-gut furrow was very weakly formed and in 18/204 embryos from mutant females, did not form at all (figure 5i). In wild-type embryos the anterior mid-gut furrow forms mid-way between the anterior tip and the cephalic furrow (figure 5d). Later the mutant embryos formed abnormal and highly reduced cephalic structures during head involution.

3.2e *mel 5*: Two alleles *mel* 5^1 and *mel* 5^2 with similar phenotypes were recovered and studied. However unlike the other *mel* loci described above, *mel 5* had incomplete penetrance. In mutant female × wild-type male matings, around 20% of the embryos hatched into normal larvae and developed into normal adults. This percentage increased in older females. In selfed crosses the percentage of normal embryos ranged from 5–12%, and varied with female age and crowding in culture bottles. Among the unhatched fraction, embryos arrested at various stages of syncitial and cellular blastoderms were observed.

3.2f *Mel 6*: One semidominant allele was recovered at this locus. Its semidominant maternal effect was established in the following way.

In the cross *Mel 6/Cy* females × +/+ males, the F_1 embryonic lethality was 24·3%. Of these embryos, 4–5% expressed the mutant phenotype. The rest of the embryos were either unfertilized or had early lethality. *Mel 6/Df(2L)E55* females × +/+ males, however, showed 95–98% embryonic mortality, with 83–85% embryos exhibiting the mutant phenotype. *Mel 6* failed to complement all the other *mel* loci in this region and females *trans* heterozygous for *Mel 6*/any other *mel* behaved like *Mel 6/Cy* females mated to +/+ males. Males of the genotype *Mel 6/Cy* or *Mel 6/Df* mated × +/+ females showed 9·8 and 19·6% embryonic lethality with no mutant embryos, establishing beyond doubt that *Mel 6* was a maternally determined semidominant embryonic lethal.

Cuticle: The mutant embryos had a broad open lacerated head with no differentiation. The chitinous head skeleton was an undifferentiated club-shaped mass. All thoracic abdominal segments were weakly differentiated (figure 3f). The embryonic pattern phenotype was fully penetrant.

3.2g *Mel 7*: This was the second semidormant *mel* isolated in this study.

Cuticle: *Mel 7* embryos showed the 'twisted' embryonic phenotype similar to mutant alleles of *dorsal* (Nusslein-Volhard 1979). The ventral ectodermal bands were poorly developed and did not extend laterally to their fullest extent. The posterior spiracles were displaced, and the head skeletal structures were reduced (figure 3g).

3.2h *Ovarian tumour* (*ovt*): One of the "female steriles", when hemizygous against *Df (2L) E55*, was found to be agametic. The females of the genotype *dp b cn*/Df (2L) E55* mated to wild-type males did not lay any eggs, although they were fertilized as judged by motile sperm in the spermathecae. Males of the same genotype were, however, fertile.

When ovaries of the hemizygous female were examined, they were found to contain two types of ovarioles. The first category contained a large number of small nuclei which stained deeply and appeared disorganised (figure 6). The second type

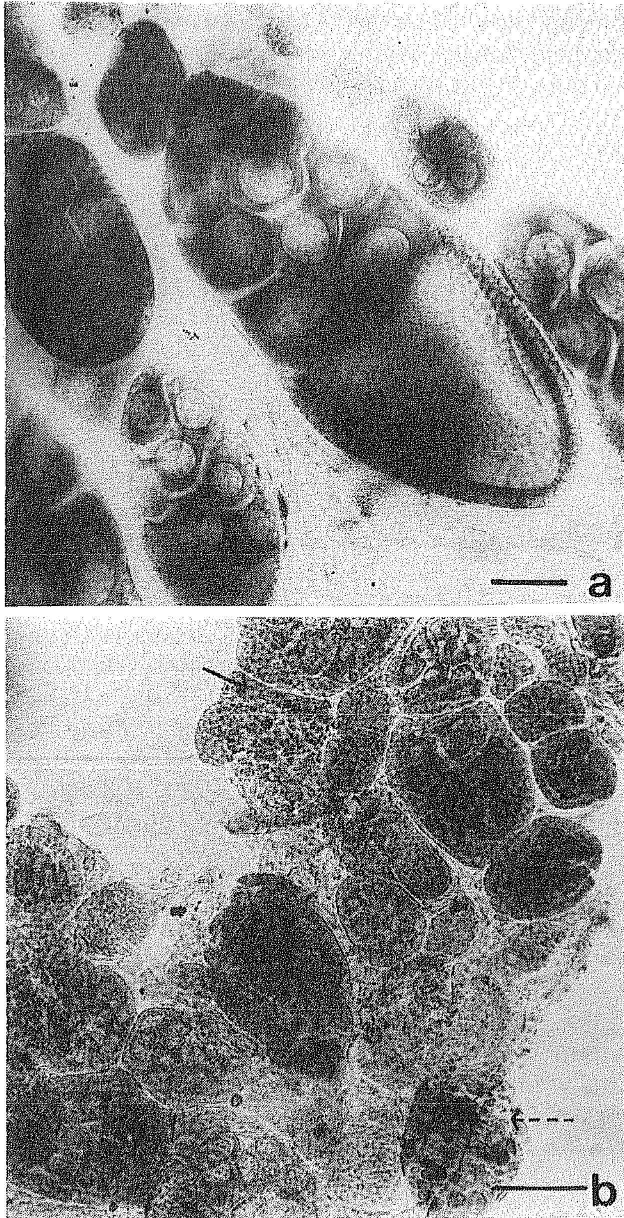

Figure 6. Aceto-orcein stained whole mount preparations of ovaries from (**a**) 4-day old Canton S wild-type female, showing normal ovariolar organization, and (**b**) 10-day old *ovt/Df (2L) E55* female. Note the absence of normal ovarioles and their tumorous nature (→). At the bottom right in (b) is an agametic ovariole (-→), with its tubular epithelial sheath. The bulbous germarium lies at its base. (Bar represents 40 μm).

of ovarioles appeared agametic and lacked oocytes. Only primary cystocytes could be seen. The occurrence of stage 14 oocytes was extremely rare.

This is, therefore, a new recessive ovarian tumour locus in *Drosophila* and is designated *ovt*. The *dp b ovt cn* mutant carrying chromosome also happened to be carrying a *cis* associated lethal since the chromosome could not be recovered as homozygous, although *dp b ovt cn/Df (2L) E55* females were viable. The developing oocytes of hemizygous females, with a very low frequency, reached maturity and were laid as immature, fragile stage 14 oocytes, which however never hatched.

4. Discussion

The results of the saturation mutational analysis of a maternally haplo-insufficient region of the *Drosophila* genome, cytologically defined by the breakpoints of the deficiency *Df(2L) E55*, has revealed the presence of 7 *maternal effect embryonic lethal* (*mel*) loci, one *ovarian tumour locus* (*ovt*) and two alleles of the gene *pads*. The screening procedure did not allow recovery of zygotic lethals within the deficiency and hence, no alleles of the zygotic lethal *spitz* mapped in this region were recovered.

It has been reported previously that several genomic regions in *Drosophila* when in hemizygous condition in females, are unable to support normal embryogenesis (Garcia-Bellido and Moscoso del Prado 1979; Garcia-Bellido *et al.* 1983). This indicates that they probably uncover maternally required genes. The identification of 7 *mel* and 1 *ovt* genes from this study, in one such region – represented by *Df (2L) E55*, confirms this presumption. Since one of the loci (*ovt*) affects ovarian organisation while the others affect embryogenesis at various steps, often leading to embryonic cuticle pattern abnormalities, the *E55* region appears to be developmentally indispensable for ovarian and embryonic organisation. A similar analysis of other maternally haplo-insufficient regions will help identify more such loci.

The next question asked was whether the *mel* genes could possibly be assigned a developmental role on the basis of their mutant phenotypes. Careful observations of mutant phenotypes of an allelic series can provide considerable clues to the function of the wild-type gene product. Different types of mutations at a locus can yield different alterations in gene activity with proportionately different phenotypes. The commonest mutations are *amorphs* or *hypomorphs* resulting in complete or partial loss of gene function respectively. Mutations leading to altered phenotypes – *Neo* or *Antimorphs*, cannot be easily interpreted to assess wild-type function. It is important to analyse multiple rather than single mutations at a locus. It is also important to identify the phenotype associated with the loss of function allele since it provides maximum information on wild-type function.

Multiple alleles were obtained at four loci – *mel 2*, *mel 3*, *mel 4* and *mel 5*, which are discussed.

4.1 *mel 2*

Out of the 3 alleles, *mel* 2^1 and *mel* 2^2 had deletions of the head cuticle structures and reduction in abdominal bands. As expected, the early embryonic events leading to the formation of the head were weak and abnormal. On this basis the function of

mel 2 appeared to be required in the formation of the head. However, the embryos of the *mel* 2^3 allele which was recessive to *mel* 2^1 and *mel* 2^2 was blocked prior to cellular blastoderm formation. The *mel* 2^3 appeared to be an *amorph*–since embryos of *mel* 2^3/*mel* 2^3 females were identical to those laid by *mel* 2^3/*Df.* (*2L*) *E55*. It was, therefore, more appropriate to conclude that *mel* 2^3 was required for blastoderm cellularization.

4.2 *mel 3*

Three alleles *mel* 3^1, *mel* 3^2 and *mel* 3^3 were recovered. The embryos of either *mel* 3^1/*Df* (*2L*) *E55* or *mel* 3^1/*mel* 3^1 females did not develop beyond a few zygotic divisions. The weaker alleles *mel* 3^2 and *mel* 3^3 completed embryogenesis but had deletions of the abdominal segments in an allele specific manner. It was concluded that *mel* 3^2 and *mel* 3^3 were *hypomorphs* because their phenotype in *trans* over the *Df* (*2L*) *E55*, or over *mel* 3^1 was more extreme than when in homozygous condition. The *mel* 3^1 allele was concluded to be an amorphic allele because its homozygous and hemizygous phenotypes were identical, and it was recessive to *mel* 3^3 and 3^2.

Since pattern defects were seen only in hypomorphic alleles, while the *amorph* had a very early block, it demonstrated that the wild-type function of *mel 3* was required very early and was general. However, the effects of the hypomorphic mutations on abdominal segments and pole cell formation indicated that the abdominal embryonic region was more sensitive than the rest of the embryo to reduced levels of the gene product. Between the two alleles *mel* 3^2 and *mel* 3^3, the latter was more extreme in abdominal deletions and also had head deletions. It is possible that the abdomen is most sensitive to a loss of *mel 3* function followed by the head, and finally in an amorphic state of *mel 3*, the zygote stops dividing after a few rounds of mitosis.

4.3 *mel 4*

The two alleles of *mel 4* did not yield enough information because both appeared to be hypomorphs and no amorphic allele could be identified. On the basis of the phenotypes of *mel* 4^1 and *mel* 4^2 it was tentatively concluded that this locus was needed in the formation of the head structures.

4.4 *mel 5*

The two non-complementing mutations recovered in this group had incomplete penetrance. All the dead embryos were pre-gastrula embryos and, therefore, it was concluded that *mel* 5^+ was required for blastoderm formation.

More detailed analysis of *mel 1*, *Mel 6* and *Mel 7* needs to be carried out before conclusions about their function can be made.

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