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A Pupal Lethal Mutation with a Paternally Influenced Maternal Effect on Embryonic Development in *Drosophila melanogaster*

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The maternal effect and zygotic phenotype of *l(1)pole hole (l(1)ph)* is described. *l(1)ph* is a zygotic lethal mutation which affects cell division of adult precursor cells in *Drosophila* larvae. The locus is located in 2F6 on the salivary gland chromosome map and four alleles have been characterized. Germ-line clonal analysis of amorphic alleles indicates that *l(1)ph* has a maternal effect lethal phenotype. Two lethal phenotypes are observed among embryos derived from female germ-line clones homozygous for amorphic alleles dependent upon the zygotic activity of $l(1)ph^+$ introduced via the sperm. *Class 1:* If no wild-type dose of the gene is introduced, embryos form abnormal blastoderms in which nuclear migration and cell formation is disrupted leading to an ill-defined cuticular pattern. *Class 2:* If a wild-type copy of the gene is introduced, blastoderm cells do not form beneath the pole cells (the pole hole phenotype); subsequently such embryos are missing cuticular structures posterior to the seventh abdominal segment (the torso phenotype). When the zygotic activity $l(1)ph^+$ is modulated using position effect variegation a new phenotype is observed among class 2 embryos in which torso embryos are twisted along their longitudinal axis.

INTRODUCTION

Early embryonic development requires not only stage- and region-specific gene expression, but also a complex pattern of gene activity during oogenesis (review in Konrad *et al.*, 1985). Most analyses of these maternal contributions have derived from studies of female sterility loci with a maternal effect lethal (MEL) phenotype (Rice, 1973; Gans *et al.*, 1975; Mohler, 1977). Many of the MEL loci affect global patterns of embryonic determination along the anterior–posterior (Nusslein-Volhard, 1979; Lohs-Schardin, 1982) or dorsal–ventral (Anderson and Nusslein-Volhard, 1984) axes. The number of female sterility loci producing MEL phenotypes is not large and may represent only 1% of the genome (see Mahowald *et al.*, 1984).

However, both molecular analyses of the complexity of RNA in oocytes (Thomas *et al.*, 1981; Davidson, 1976) and observations that many essential loci (i.e., mutations which result in lethality at some stage in the life cycle) show a maternal effect (Ripoll, 1977; Ripoll and Garcia Bellido, 1979) clearly suggest that a large portion of the genome contributes information during oogenesis essential for normal developmental processes. Recently, the availability of a dominant female sterile mutation on the X-chromosome (Busson *et al.*, 1983; Perrimon, 1984) has made it possible to examine the maternal effect of essential genes by inducing a germ-line clone of a lethal mutation and determining the phenotype of the embryos derived from such clones. Using this methodology Garcia-Bellido and Robbins (1983) and we (Perrimon *et al.*, 1984a) have shown that normal gene activity of about 80% of lethal loci is required during oogenesis. A small fraction of these loci exhibit specific MEL on embryonic development (Perrimon *et al.*, 1984a). In this study we report a detailed characterization of one of them.

Lack of $l(1)pole\ hole^+$ gene activity disrupts cell division in imaginal discs and also produces a specific maternal effect lethal phenotype in embryos derived from germ-line clones. This phenotype is indistinguishable from the torso (Nusslein-Volhard *et al.*, 1982) embryos produced by a number of female sterility loci. Furthermore, the embryonic phenotype is partially rescued by providing wild-type gene activity in the zygote. We have called the locus *lethal(1) pole hole (l(1)ph)* because of a defect in the posterior blastoderm.

MATERIALS AND METHODS

Strains. Four *l(1)pole hole* alleles were obtained from Dr. G. Lefevre (*EA75, DF903, DC817,* and *C110*) and maintained either in attached-X stocks covered by $Dp(1;Y)w^{+803}$ or as an *FM7* balanced stock. *C110* was X-ray induced; the other three alleles, ethyl methane sulfonate (EMS) induced.

In this study we used one deficiency: *Df(1)64c18* (Df(1)2E1-3C2 area, Craymer and Roy, 1980), and two duplications: $Dp(1;Y)w^{+803}$ (Dp(1;Y)2D1-2; 3D3-4, G.

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Lefevre, personal communication) and *Dp(1;3)w^{vco}* (Dp(1;3)2B17-C1;3C4-5;77D3, Lindsley and Grell, 1968).

The recessive male sterile mutation *ms(3)K81* was obtained from Dr. Y. Fuyama and is kept in a *TM3, Sb, Ser* balanced stock.

The dominant female sterile *Fs(1)K1237* is maintained in an attached-X stock: *C(1)DX, y f/Y* females crossed with *K1237 v^{24}/Y* males. For a more detailed description of other stocks used, see Lindsley and Grell (1968).

Germ-line clones. Germ-line clones were induced using the dominant female sterile technique as previously described by Perrimon and Gans (1983) and Perrimon *et al.* (1984a). A constant dose of 1000 rad was administered at the end of the first larval instar stage (General Electric, 100 kV, 5 mA, 3'35", 1-mm aluminium filter). Between 5 and 10% of females carrying a germ-line clone were recovered.

Embryo morphology. Embryos were examined 24 hr after egg laying by the Hoyer's mount technique of van der Meer (1977). Histological sections of embryos were prepared as described by Mahowald *et al.* (1979). Eggs were collected for 2 hr and aged 2 hr before fixation.

Scanning electron micrographs. Micrographs of adult eyes were prepared as described by Bryant (1979). Relative eye sizes were determined by counting the number of ommatidia per compound eye on appropriate SEM photomicrographs of *C110* and wild-type (*Oregon R-P2*) males.

Determination of effective lethal phase. Heterozygous *l(1)ph/+* females mated to *+/Y* males were allowed to lay more than 100 but less than 600 eggs in shell vials. The eggs were counted, aged 24 hr, and the number of unhatched embryos was determined. Samples of unhatched eggs were examined for evidence of embryonic development. A minimum of 100 larvae were transferred to fresh cultures and observed for larval lethality. The number of pupae and emergent adults were counted. Dead pupae were examined and the relative stage (early, middle, or late) of mortality was determined. The phenotype and sex of all adults were recorded.

Search for diploid cells. Search of diploid structures (imaginal discs, imaginal ring of salivary glands, and gonads) in whole mounts of late third-instar larvae was carried out using Hoechst fluorescence labeling of nuclei. Larvae were dissected in a fresh Ringer's (Ephrussi and Beadle, 1936) solution. The structures to be observed were transferred and spread on slides coated with poly-L-lysine (1 mg/ml from Sigma). The Ringer solution was gently removed and tissues were incubated for 5 min in a drop of Hoechst-33342 (1 μg/ml dissolved in Ringer solution). Tissues were successively washed in Ringer's for 1 min, acetone fixed at −20°C for 5 min, washed at room temperature, and mounted in a solution of 90% glycerol. Slides were inspected under epifluorescence illumination.

Whole brains were dissected from third-instar larvae treated 2 hr in colchicine and prepared individually on slides to examine mitotic chromosomes as described by Guest and Hsu (1973).

RESULTS

The first allele of *l(1)ph* (*EA75*) was found during our study of the maternal effect of lethal mutations (Perrimon *et al.*, 1984a) and three additional alleles have subsequently been identified [*DC817, DF903,* and *C110* (Perrimon *et al.*, 1984b)]. The locus maps to position 2F6 on the cytogenetic map and is separable from distal adjacent zygotic loci by three breakpoints: *Df(1)Pgd kz, Df(1)X12,* and *Df(1)JC19* and from the proximal zygotic lethal locus (*giant*) by two breakpoints *Df(1)62g18* and *Df(1)TEM75* (Perrimon *et al.*, 1984b; unpublished observations).

The Zygotic Lethal Phase of l(1)ph Is at Larval-Pupal Interphase

The three EMS-induced alleles (*EA75, DF903,* and *DC817*) show a lethal phase at the larval-pupal boundary whereas *C110* hemizygotes have a pupal-adult lethal phase (Table 1, Results concerning the analysis of *DF903* and *DC817* have been omitted from Table 1 because these alleles are indistinguishable from *EA75*). A classical method to determine whether or not a mutant allele has any residual gene activity is to compare the lethal phase of each allele in the homozygous condition with that found when heterozygous with either a deficiency for the locus or with different strength alleles (Table 1). The three EMS-induced alleles, heterozygous for a deficiency (*Df*) of the locus, exhibit the same zygotic lethal phases as the homozygous controls. However, nearly all *C110/Df* females die as pupae and none emerge. Heteroallelic combinations of *C110* with each of the EMS-induced alleles were checked both for potential complementation and for partial genetic activity. In these experiments females carrying amorphic *l(1)ph* alleles were crossed with *C110* males carrying a duplication, and the zygotic lethal phases of females (selected at early third-instar stage by their smaller gonad size) were determined. Nearly half of the female larvae die as pupae (none emerge) which corresponds to the lethal phase of *C110/Df* females. However, most transallelic females (*C110*/EMS-induced alleles) appeared to die as late pupae, whereas *C110/Df* females died as both early and late pupae. This result suggests that *EA75, DF903,* and *DC817* are not completely amorphic. It is possible,

TABLE 1
ZYGOTIC LETHAL PHASE OF *l(1)pole hole* ALLELES

Cross	*T* (α)	*N*	Lethal phase of progeny (%)				Emerging progeny (%)		*f*(%)
			L3	EP	LP	A**	♀	♂	
EA75/+ × Df/DpY	25	127*	7	44	0	0	49	0	
EA75/+ × EA75/DpY	25	76*	3	41	0	0	56	0	
C110/+ × Df/DpY	25	403*	1	24	27	0	48	0	
C110/+ × C110/DpY	25	201*	0	3	15	30	52	0	
EA75/+ × C110/DpY	25	73*	4	0	44	0	52	0	
EA75/+ × +/Y	20	1645	4	22	0	0	44	20	14
	25	231	13	18	0	0	46	23	41
	29	580	14	6	0	0	56	24	71
C110/+ × +/Y	20	1129	0	3	6	14	51	26	35
	25	675	0	1	11	12	56	20	49
	29	315	0	2	7	14	50	27	40

Note. Three sets of crosses were performed: Heterozygous females for a *l(1)ph* allele were crossed to: (1) *Df(1)64c18/Dp(1;Y)w*$^{+303}$ males (*Df/DpY*) or to the same *l(1)ph* allele, (2) *C110/Dp(1;Y)w*$^{+303}$ males (*C110/DpY*), and (3) wild-type males (*+/Y*) at three different temperatures (20, 25, and 29°C). Cross (1) indicates the zygotic lethal phase of *l(1)ph* alleles and the nature of the mutations (null or hypomorph), cross (2) the zygotic lethal phase of *l(1)ph* alleles in heterozygous combination with the hypomorphic allele *C110*, and cross (3) the temperature sensitivity of the zygotic lethal phase of males hemizygous for two *l(1)ph* alleles. The number of larvae (*N*) transferred to fresh vials after hatching, and the stage of mortality (third instar stage, L3; early pupa, EP; late pupa LP; and emerging adult, A) are shown. The number of emerging wild-type adults (females and males) are given. Appropriate markers were used in each set of crosses to identify each class of progeny. Finally, we have calculated the relative early lethal phase frequency (*f*%) for *EA75* and *C110* from the formula: $f\% = N(\text{L3})/N(\text{L3}) + N(\text{EP}) \times 100$ for *EA75* and $f\% = N(\text{EP}) + N(\text{LP})/N(\text{EP}) + N(\text{LP}) + N(\text{A}) \times 100$ for *C110*. An asterisk in the column of larvae transferred indicates that only female larvae were transferred (selected by their small gonad size). Two asterisks in the column of dead progeny refer to *C110* hemi- or homozygous progeny identified by their eye phenotype and early adult mortality.

however, that the deficiency contains other genes whose hypodosage reduces viability. Since the first series of crosses showed no evidence for genetic activity in the EMS-induced alleles, we assume that they are either amorphic (null) or nearly amorphic mutations of the gene while *C110* is clearly a hypomorphic (reduced activity) mutation. Thus, these crosses suggest that the EMS-induced alleles behave as amorphic mutations of the gene while *C110* acts as an hypomorphic mutation. In addition, these crosses establish that there is no intraallelic complementation between *C110* and the other *l(1)ph* alleles. Similarly no intraallelic complementation was observed between the EMS alleles.

Because of the usefulness of temperature-sensitive alleles for the analysis of expression of genes, we examined the lethal phase of the *l(1)ph* alleles at three temperatures (Table 1). A slight temperature effect can be detected in that the number of mutant larvae that pupated is greater at low temperatures for *EA75*, *DF903*, and *DC817* (compare the value of "*f*" in Table 1). The *C110* allele shows no temperature effect.

l(1)ph Shows a Small Disc Phenotype

Late third-instar larvae of the EMS-induced alleles do not have any apparent major imaginal discs following dissection and observation under the dissecting microscope. However, rudimentary imaginal discs (Fig. 1a) can be detected with Hoechst staining, indicating that the lack of *l(1)ph* activity results in a small disc phenotype (Shearn, 1978). Similarly, the size of the imaginal ring in the salivary gland (Fig. 1c) and gonads (Fig. 1e) is reduced. Finally, whereas an average of 254 mitotic figures were found in squashes of 4- to 5-day-old wild-type larval brains, an average of only 25 were found in *EA75* larvae of comparable developmental stages. These results provide evidence that this locus is essential for continued cell proliferation of diploid cells in the larvae. Reduced cell proliferation is also indicated by the phenotype of adults hemizygous for the hypomorphic allele (*C110*) which has reduced disc size. For example, the number of ommatidia in *C110* emerging males is reduced by 30% from wild type. Such *C110/Y* males did not exhibit any other obvious morphological abnormalities. We found an average of 714 facets in two wild-type and 460 in three *C110* eyes. This phenotype is more extreme in *C110/Df(1)64c18* females in which we counted about 200 facets.

In summary, analysis of zygotic lethal phases establishes that *l(1)ph* is a "small disc" mutation and that three alleles appear to be amorphic and one hypomorphic. Since the temperature effect on pupariation

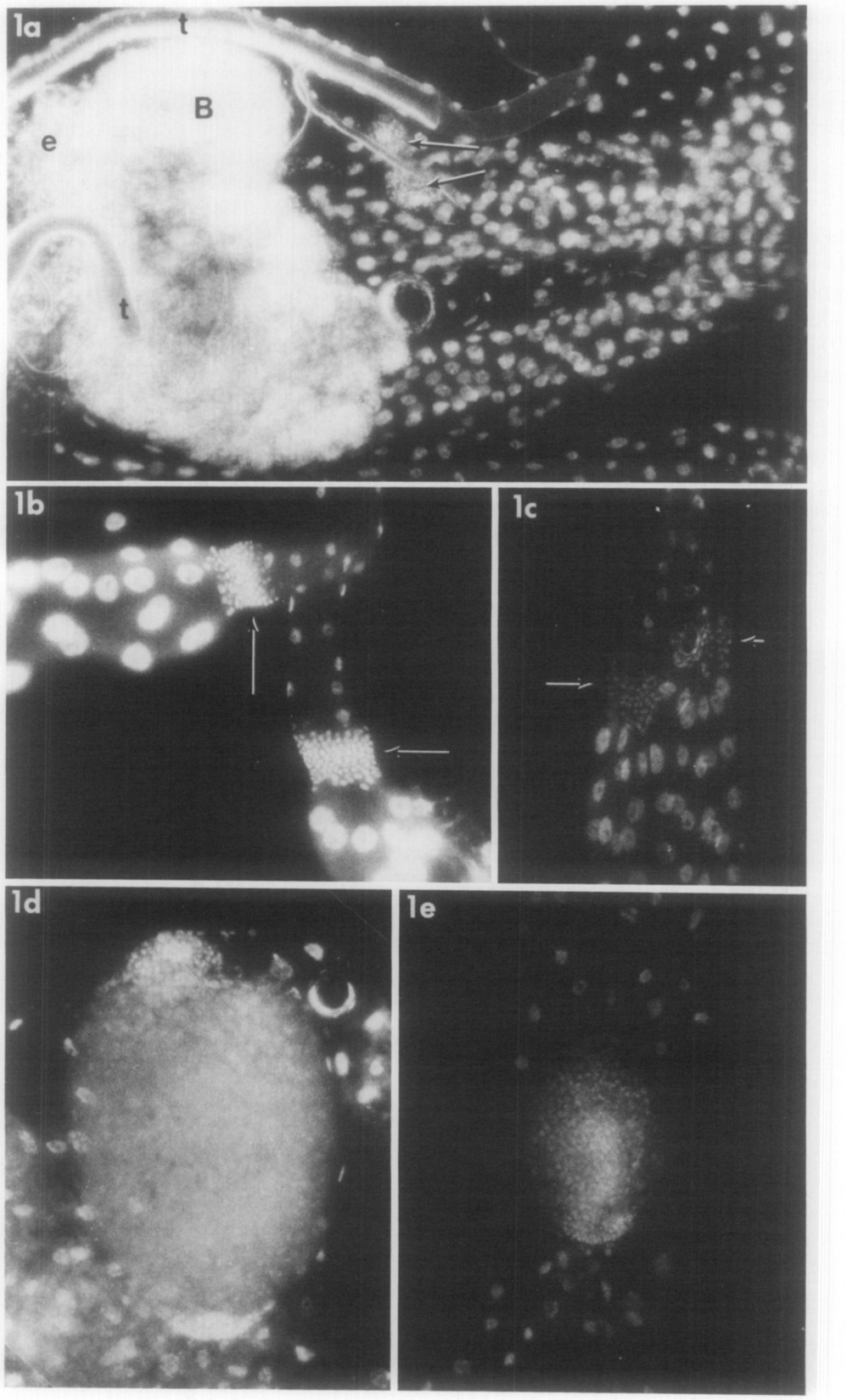

FIG. 1. Imaginal structures of hemizygous *EA75* larvae analyzed by Hoechst fluorescent staining. (a) Is a whole mount of a third instar *l(1)ph* larval brain (B) with associated trachea (t) and two unidentified imaginal discs (arrows). The small masses (e) probably are greatly reduced eye discs. Whole mounts of the imaginal rings of the salivary glands (arrows) of wild type (b) and *l(1)ph* (c) are shown. Note the reduced number of cells in the *l(1)ph* rings. The salivary gland nuclei are nearly equal in size although they may be slightly reduced in *l(1)ph*. The apparent reduced fluorescence in (c) is due to variation in technique. Whole mounts of mature larval testis of a wild type (d) and *l(1)ph* (e). ×720 magnification.

as well as the delay in pupariation (results not shown) are not allele specific, it is likely that these properties are common features of the discless phenotype, as previously described for other "discless" mutations (Szabad and Bryant, 1979).

l(1)ph Mutations show a MEL Phenotype in Germ-line Clones

Previous work (Perrimon *et al.*, 1984a) has indicated that the *EA75* allele showed a maternal effect lethal phenotype. Germ-line clones of the remaining alleles were induced by X-ray irradiation of *l(1)ph*$^{+}$ *Fs(1)K1237/l(1)ph Fs(1)K1237*$^{+}$ larvae and the resulting flies were crossed to wild-type males. Both *DF903* and *DC817* had a maternal effect lethal phenotype while most embryos from germ-line clones of *C110* developed normally. The zygotic lethal phase of the *C110/Y* males derived from germ-line clones appeared identical to the zygotic lethal phase of control hemizygotes. While this result indicates that *C110* does not exhibit a maternal effect, a fraction of the *C110* embryos did not hatch, suggesting that a weak maternal effect might exist (see below).

Embryos derived from homozygous germ-line clones of the three EMS-induced alleles display two phenotypes (Figs. 2 and 3). One-half of the embryos exhibit a cuticular pattern that is nearly normal except that structures posterior to the seventh abdominal segment are absent (class 2 embryos, Fig. 2b). Occasionally, these embryos exhibit what appears to be a portion of the eighth abdominal segment (Fig. 2c), but spiracles, filzkorper, and anal tufts are never found. This phenotype has been termed torso (Nusslein-Volhard *et al.*, 1982) and is produced by a series of female sterility loci. They also exhibit occasional anterior head involution defects and both U-shaped and twisted phenotypes (see below). The remaining 50% of the embryos exhibit a weak disorganized cuticle (class 1 embryos, Fig. 3a) consisting mostly of dorsal structural components with only occasional ventral denticle patches (Fig. 3b) and, very rarely, spiracles with filzkorper (Fig. 3c).

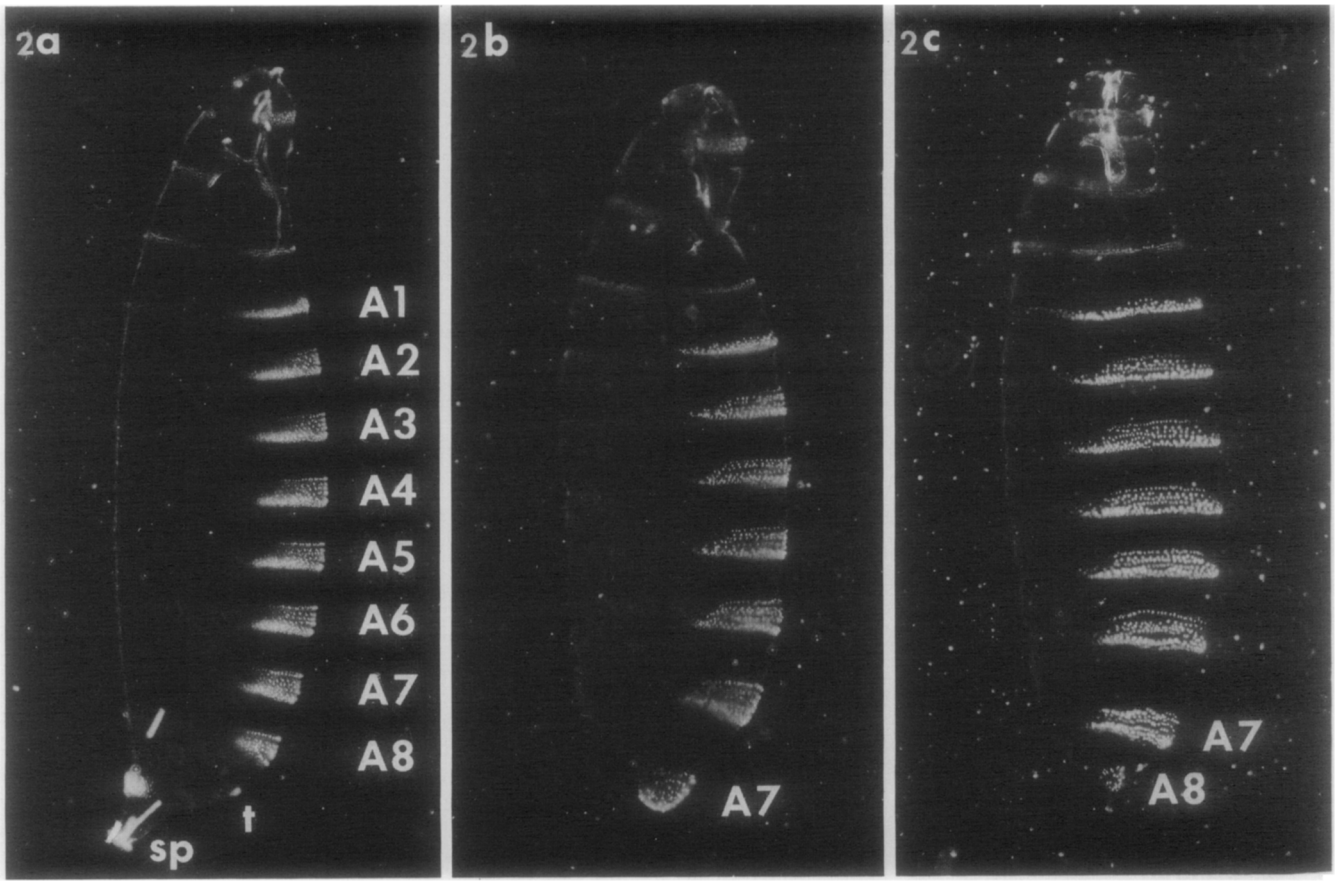

FIG. 2. Phenotype of germ-line clone embryos (class 2). Dark field of a wild-type embryo (a) showing the eight abdominal segments (A1 to A8) the anal tuft (t), and the spiracle (sp). In torso embryos (b) structures posterior to A7 are missing. About 5% of these possess part of A8 (c). ×270 magnification.

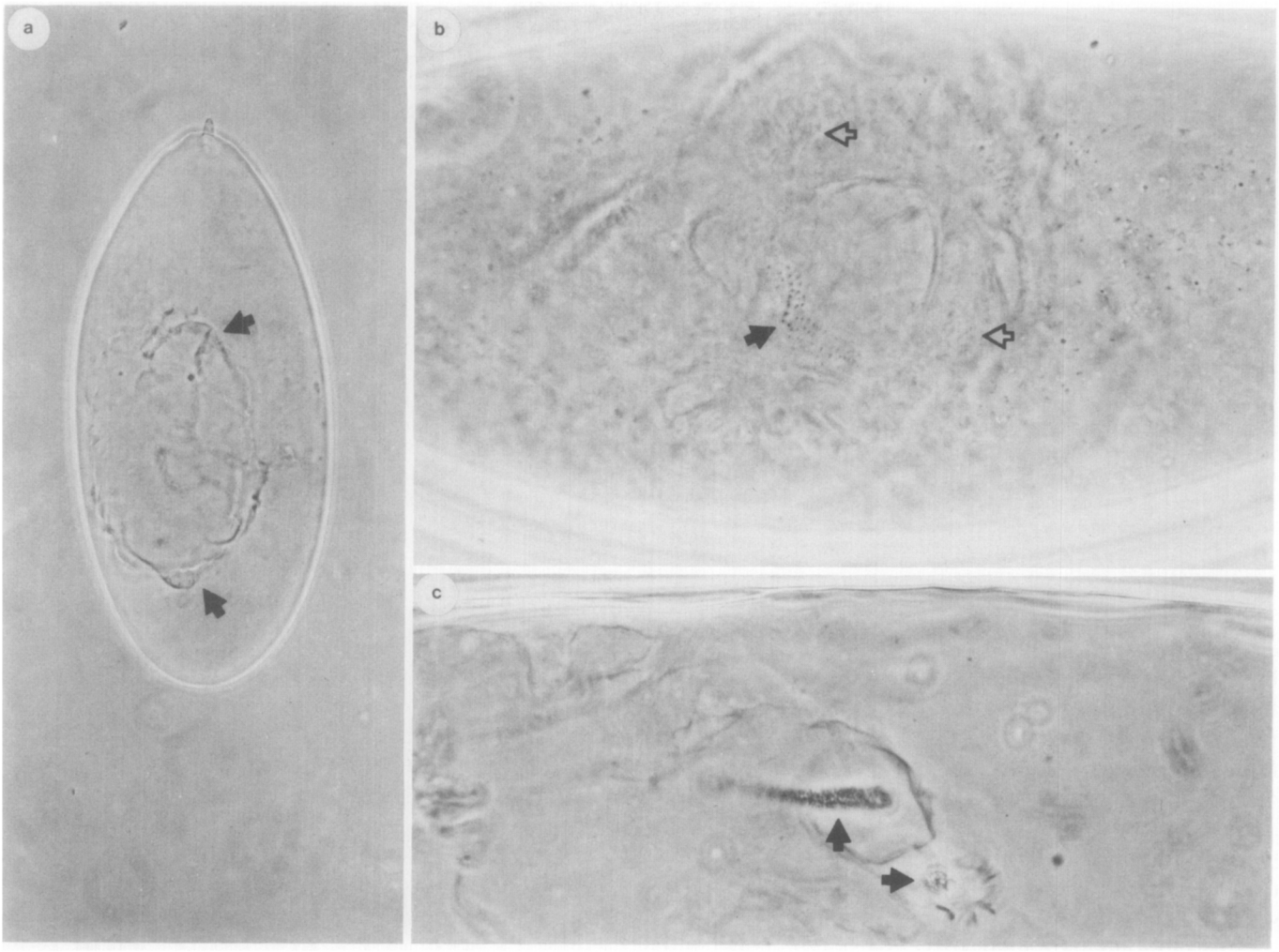

FIG. 3. Phenotype of germ-line clone embryos (class 1). Half the embryos derived from germ-line clones of *EA75*, *DF903*, and *DC817* present an ill-defined cuticular pattern (arrows in a) which consists mostly of dorsal cuticle (open arrows in b); however, rare small patches of ventral cuticle (closed arrow in b) are found. In less than 1% of the embryos, filzkorper material (arrows in c) is evident. a = ×225; b and c = ×405 magnification.

The Torso Phenotype is Due to Wild-Type Gene Activity Introduced with Sperm

The fact that embryos derived from homozygous germ-line clones of the EMS-induced *l(1)pole hole* alleles (when females were crossed to $+/Y$ males) distributed equally between the two classes suggested that the two classes of embryos represented the different sexes. This hypothesis was tested by crossing females containing germ-line clones of the *EA75* mutation to genetically different classes of males (Table 2). When crossed with males carrying a deficiency on the X and a duplication on the Y chromosome of the chromosome region, the same ratio of class 1 and 2 embryos is found as when crossed with wild-type males. However, when crossed with wild-type males carrying a duplication on the Y ($Dp(1;Y)w^{+303}$), all embryos exhibit the torso (class 2) phenotype. These results indicate that the two embryonic phenotypes reflect either the introduction of a wild-type dose of the gene via sperm (class 2) or the absence of a wild-type dose in the zygote (class 1) and that male and female embryos behave the same way with respect to this partial zygotic rescue. It is also possible to generate embryos, derived from germ-line clones, that have two wild-type doses of the *l(1)pole hole* gene. In the fourth cross in Table 2, two wild-type doses are introduced using a duplication of the gene on the third chromosome $Dp(1;3)w^{vco}$, and a ratio (3 to 1) of class 2 and class 1 embryos was obtained. Since no additional rescue beyond the torso (class 2) phenotype was detected among the embryos, we conclude that the zygotic rescue by one dose is equivalent to that of two doses. Thus, the rescue does not show a dosage effect.

TABLE 2
GERM-LINE CLONE ANALYSIS OF *EA75*

Genotype of males	Genotype of embryos	Genotype of embryos	*N*	Nu	*N* class 1	*N* class 2	Expected class 1	Expected class 2
+/Y	*EA75/Y*	*EA75/+*	1406	263	547	596	571.5	571.5
Df/DpY	*EA75/DpY*	*EA75/Df*	112	23	40	49	44.5	44.5
+/DpY	*EA75/DpY*	*EA75/+*	234	71	0	163	0	163
+/Y; +/Dp3	*EA75/Y; +/Dp3* *EA75/Y; +/+*	*EA75/+; +/Dp3* *EA75/+; +/+*	292	16	59	217	69	207
+/Y; ms/ms	*EA75*	*EA75*	128	97	31	0	31	0

Note. Females possessing homozygous germ-line clones of *EA75* were crossed with five genetically different males. The resulting genotype of the male and female embryos derived from each of these crosses is shown. The eggs studied (*N*) were classified according to their phenotype in Hoyer's mounts: Unfertilized (Nu), class 1 (weak, ill-defined cuticle, see Fig. 3) and class 2 (torso embryos, Fig. 2b). The expected numbers of class 1 and class 2 embryos assuming the torso embryos correspond to those which did not received a wild-type dose of *l(1)ph* via sperm and those which did, respectively, are shown. Symbols: see legend Table 1, $Dp(1;3)w^{vco}$ is referred to as *Dp3* and *ms(3)K81* as *ms*. The observed and expected frequencies are not significantly different ($P < 0.05$).

As a final demonstration that the two classes of embryos observed depends upon the paternal contribution, we crossed females carrying a germ-line clone for the *EA75* mutation with males homozygous for the recessive male sterile mutation *ms(3)K81* (Table 2). Males homozygous for *ms(3)K81* produce sperm which are incapable of syngamy but which are capable of activating the egg; about 20% of such activated eggs lead to haploid embryos which die before hatching (Fuyama, 1984; results not shown). When crossed to such males, developing eggs laid by females carrying a germ-line clones homozygous for *EA75* should only exhibit the class 1 phenotype. This was indeed observed: (Table 2) no class 2 embryos were found, confirming our previous results.

The Phenotype of Blastoderm-Staged Embryos

Embryos derived from germ-line clones homozygous for amorphic alleles of *l(1)ph* were examined histologically at the blastoderm stage. Although it is difficult to collect large quantities of accurately staged embryos derived from germ-line clones because flies possessing clones are quite rare and have a reduced egg production, we have identified two distinct blastoderm-stage phenotypes. Embryos which we interpret as the blastoderm stage for class 2 torso embryos (Figs. 4a, b) possess a nearly normal cellular blastoderm including pole cells with the exception of a small uncellularized region at the posterior pole beneath the pole cells. This pole hole phenotype and the terminal torso phenotype (Fig. 2b) are nearly identical to phenotypes observed in five maternal effect lethal mutations distributed on the major chromosomes (Konrad *et al.*, 1985; A. P. Mahowald, unpublished observations; T. Schupbach, personal communication). The second blastoderm phenotype (Fig. 4c) appears to possess multiple layers of abnormal nuclei situated along the peripheral cytoplasm. The cytoplasm is distributed unevenly along the surface. Pole cells are formed, although their number appears reduced. Some cells apparently form at the periphery of the blastoderm (Fig. 4c) and these cells probably secrete the rudimentary cuticle (Fig. 3).

Weak Maternal Effect of the Hypomorphic Allele

Eighty percent of progeny derived from *C110* homozygous germ-line clones die at the same lethal phase as progeny derived from heterozygous *C110* mothers. However, approximately 20% die during embryonic development (Table 3) without any features obviously different from wild-type embryos. Moreover, when flies with homozygous *C110* germ-line clones are mated to *Df/DpY* males, half of the embryos fail to hatch (Table 3), although they have a normal cuticular pattern. These results suggest that *C110*, although hypomorphic, still exerts a maternal effect such that a portion of the offspring die during embryonic development. If this critical period is passed, then the ordinary lethal phase ensues.

U-Shaped and Twisted Phenotype

The availability of a hypomorphic allele as well as variegating duplications provides the opportunity to examine the effect of differing amounts of the $l(1)ph^{+}$ gene product on early embryonic development. We have examined the embryonic phenotypes produced

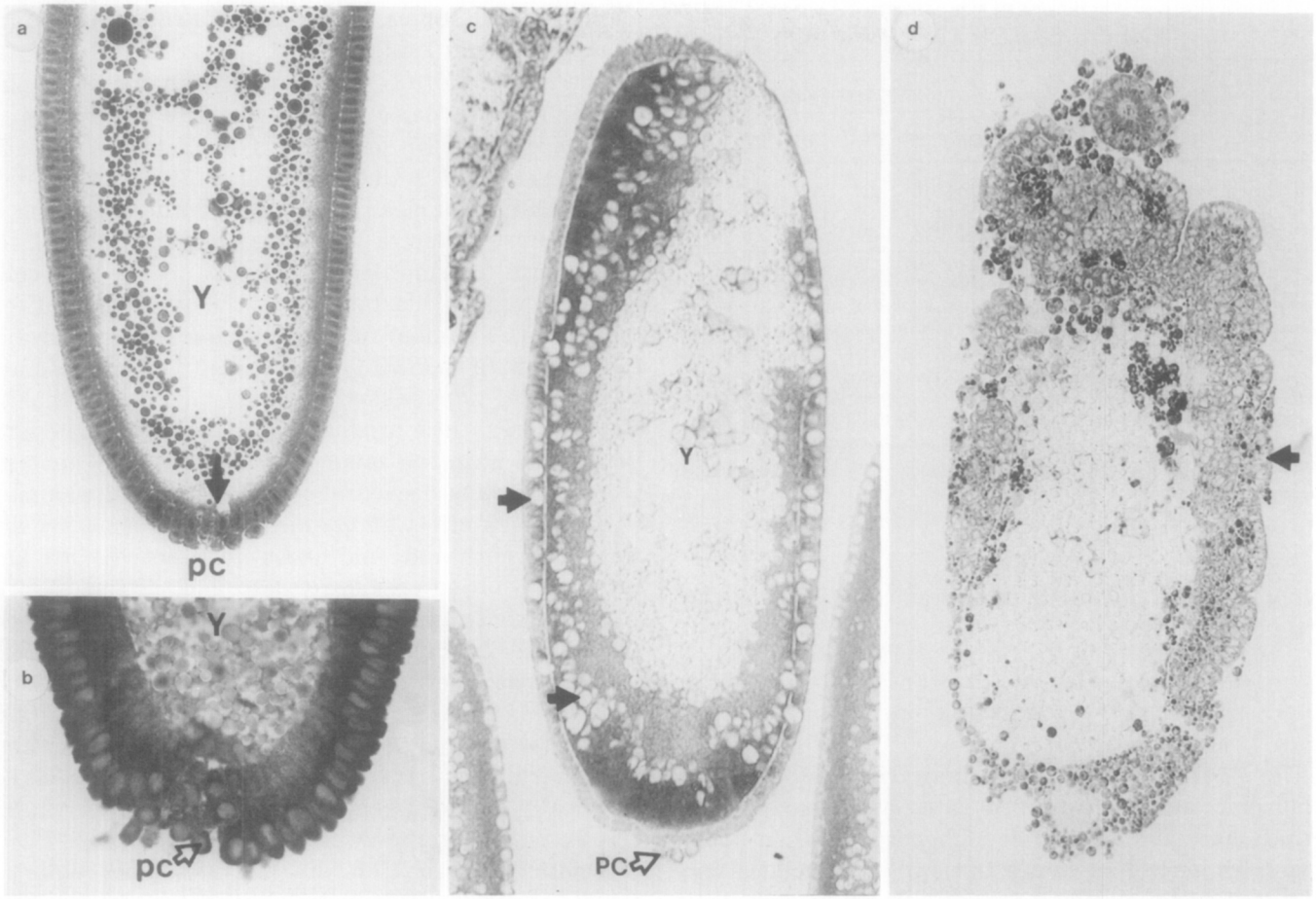

FIG. 4. Morphology of blastoderm stage *l(1)pole hole* embryos. Embryos derived from germ-line clones of null alleles of *l(1)ph* crossed to wild-type males (+/*Y*) were sectioned at 3 ± 1 hr. Two phenotypes were found. Half exhibited the pole hole phenotype (a, b, lack of blastoderm cells underneath the pole cells indicated by arrows) and the other half exhibited what we referred as a partial "acellular-multilayer" blastoderm (c). In such blastoderms, a few cells are formed (top arrow) while many nuclei do not migrate properly (bottom arrow). In latter sections abnormal embryos with some differentiated tissues are found (d). We believe (c) and (d) embryos represent Class 1 embryos (Fig. 3). Nomenclature: PC pole cells; Y yolk. (a, c, and d) ×216, (b) ×504 magnification.

when *C110/DpY* males were mated to flies with germ-line clones of the amorphic allele *EA75*. In addition we crossed such flies to +/*Y* and +/*DpY* males at three different temperatures, chosen to enhance the variegating position effect of the duplication (Table 4). Four phenotypes were observed among the embryos produced by these crosses: class 1 and 2 embryos (described earlier), a class of torso embryos which are U-shaped (Fig. 5a), and a class of twisted torso embryos (Fig. 5b). Whatever the temperature no phenotypic differences among class 1 embryos can be found. Among class 2 embryos, however, the frequency of U-shaped torso embryos increases at higher temperatures, and this phenotype is found among all zygotes possessing a wild-type *l(1)ph*$^+$ dose or a *C110* allele (Table 4). U-shaped embryos apparently fail to undergo germ band shortening and may possess somewhat more severe anterior defects than other torso embryos. This may reflect a morphogenetic interaction between the torso phenotype and the speed of embryogenesis (as temperature increases, embryogenesis proceeds faster). The twisted torso embryonic phenotype (Fig. 5b) was found only when the paternal genotype included the duplication *Dp(1;Y)w*$^{+303}$. The frequency of the twisted phenotype decreases as temperature increased. Activity of genes carried by some duplicated segments has been shown to be variable (variegation phenomena). Because variegation of gene activity decreases at higher temperature (review by Spofford, 1976), we believe the twisted phenotype is due to variegation of *l(1)ph*$^+$ carried by *Dp(1;Y)w*$^{+303}$. At the present time we cannot rule out the possibility that such embryos indicate a slight temperature sensitivity of the *l(1)ph*$^+$ gene activity. Because no major differences are observed between crosses involving *C110/DpY* and +/*DpY* males, we conclude that the reduced zygotic activity of the *C110*

TABLE 3
GERM-LINE CLONE ANALYSIS OF *C110* (AT 25°C)

				Dead progeny			Adults	
	N	Nu	Nuh	L3	P	A*	*N*	*N*+
C110/C110 × +/*Y*	910	196	142	10	247	19	296	0
C110/C110 × *Df/DpY*	360	52	162	22	36	0	0	88

Note. Females which possess homozygous germ-line clones for *C110* were crossed with two classes of males. The total number of eggs (*N*) examined, the number exhibiting no development (Nu), and the number which developed but did not hatch (Nuh) are indicated. The lethal phase of the progeny (L3 = third instar larvae, P = pupae, A = adult) and the number of wild-type adults are shown. A distinct maternal effect on embryonic development can be detected when the zygotic activity of *l(1)ph* is reduced by the introduction of a null dose (Deficiency) via sperm. Symbols: see legend Table 1. The asterisk in the column of dead progeny indicates the number of *C110/Y* males which emerged.

hypomorphic *l(1)ph* allele compared to *l(1)ph*$^+$ has very little (or no) effect.

DISCUSSION

The *l(1)ph* locus has been analyzed using 4 alleles. Amorphic alleles exhibit a late larval-early pupal lethal phase and a small disc phenotype. When homozygous in germ-line clones these alleles produce very distinctive and specific maternal effects on embryonic development. Clonally derived embryos receiving a wild-type dose of *l(1)ph* (class 2) form a normal blastoderm with the exception of an acellular region beneath the pole cells at the posterior tip. Such embryos later exhibit the torso cuticular pattern (Konrad *et al.*, 1985). Embryos not receiving a wild-type dose of the gene (class 1) are apparently able to partially cellularize the blastoderm and later to produce only an ill-defined cuticle. A variety of different embryonic phenotypes are exhibited by embryos derived from homozygous germ-line clones of amorphic *l(1)ph* alleles depending on the zygotic activity of *l(1)ph*$^+$: (1) when no zygotic activity of *l(1)ph* is present embryos exhibit mainly dorsal cuticle with the occasional presence of filzkorper and patches of ventral cuticle, (2) when some zygotic activity of *l(1)ph* is provided by manipulating the temperature in the presence of a variegating duplication, embryos frequently exhibit both the torso and a twisted phenotype, and (3) when full activity is present typical torso embryos are found. It should be noted that the introduction of two wild-type doses into the zygote did not support embryonic development beyond the torso phenotype. This lack of rescue by additional doses of the wild-type allele has previously been observed in the germ-line clonal analysis of *zw3* (Garcia-Bellido and Robbins, 1983) and *Notch* (Jimenez and Campos Ortega, 1982).

The problem of the relation between the torso phenotype and the defect in the posterior blastoderm is still unresolved. On one hand, from fate maps (Lohs-Shardin *et al.*, 1979; Underwood *et al.*, 1980; Hartenstein *et al.*, 1984) it is clear that the structures missing in torso embryos derive from the posterior 20% of the blastoderm, a region encompassing nearly 1000 cells. The defect in the blastoderm, on the other hand affects only a small number of cells varying from about 5 in *l(1)ph* to more than 25 in *fs(1)Nasrat*^{211} (Mahowald and Degelmann, unpublished observations). Nevertheless, four different loci produce the pole hole blastoderm defect and have the same torso embryonic phenotype. Further work is needed to clarify this relationship.

Loci which affect embryonic pattern formation and for which both maternal and zygotic activity are sufficient, have been previously described, as for example *Notch* (Jimenez and Campos-Ortega, 1982) and *Polycomb* (Denell, 1982; Haynie, 1983). *l(1)ph* appears, however, to represent a new class of genes in which the absence of both maternal and zygotic gene activity results in one lethal embryonic phenotype whereas the absence of maternal but the presence of various levels of paternally provided gene activity lead to different, but less extreme, embryonic lethal phenotypes.

Zygotic transcription in the *Drosophila* embryos starts at the preblastoderm stage (Zalokar, 1976;

TABLE 4
MODULATION OF THE ZYGOTIC ACTIVITY

Male	*T* (°C)	*N*	Pole hole (%)	Twisted (%)	U-shaped (%)
+/*Y**	18	128	100	0	0
	25	107	86	0	14
	29	78	76	0	24
+/*Dp(1;Y)*	18	22	77	23	0
	25	90	72	15	13
	29	24	65	5	30
C110/Dp(1;Y)	18	196	68	32	0
	25	96	75	17	8
	29	100	43	12	45

Note. Effect of temperature, the zygotic hypomorphic allele, and duplication (*Dp(1;Y)w*$^{+805}$) on the embryonic phenotype of embryos derived from homozygous *EA75* germ-line clones. The frequency of torso (Fig. 2b), twisted torso (Fig. 5a), and the U-shaped torso (Fig. 5b) embryos are shown. In the first cross (*) class 1 (Fig. 3) embryos have not been included. They are similar at 18, at 25, and at 29°C, and their frequencies are similar to the results presented in Table 2. *T* is the temperature of culture, *N* is the number of embryos scored. See text for discussion.

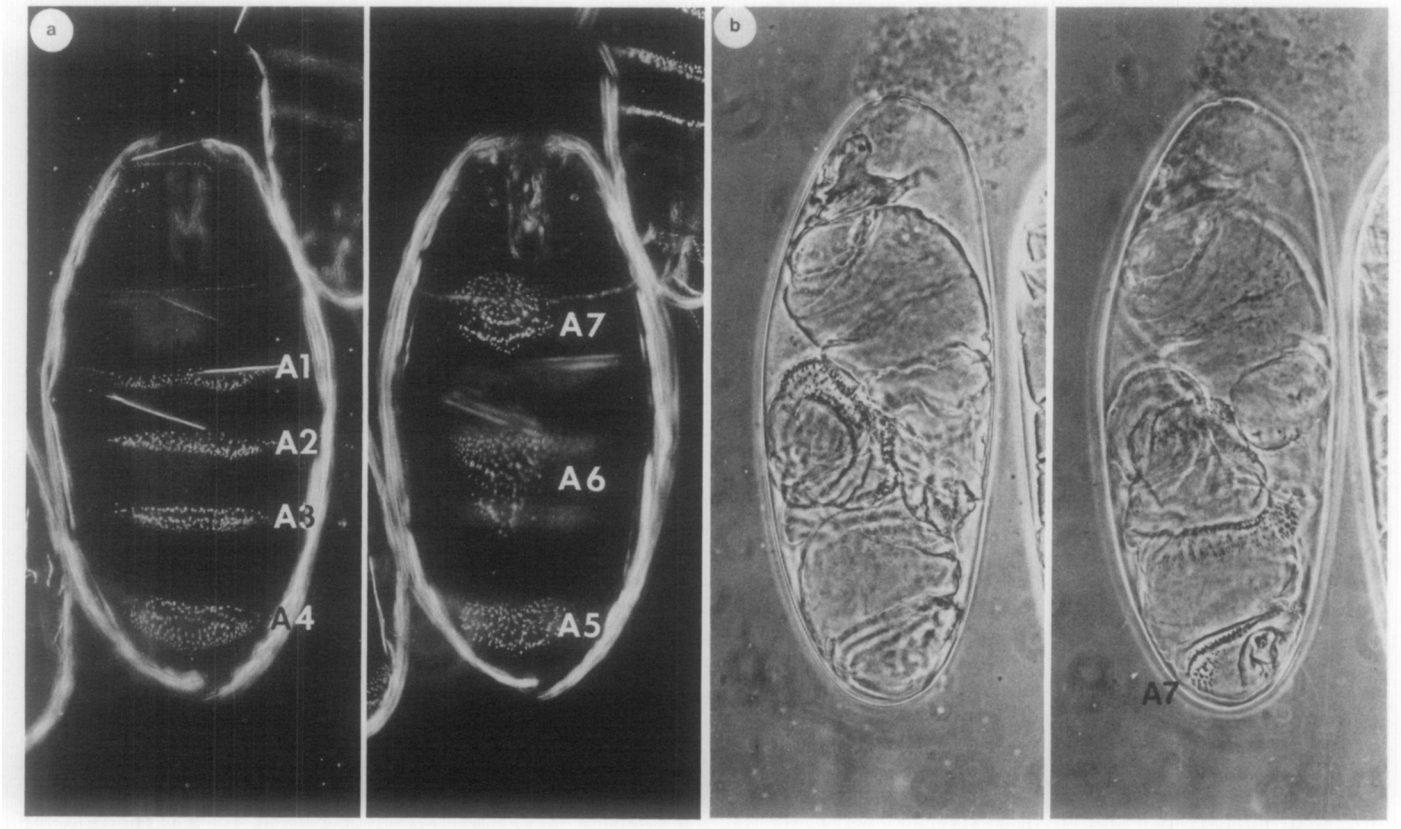

FIG. 5. Embryonic phenotypes produced by temperature and variegating duplication effects. The phenotype in the left two frames (a) represents dark-field photo of a torso embryo at two focal planes. Such embryos appear to not have undergone normal germ band shortening. They also appear to possess stronger head involution defects than other torso embryos (Fig. 2b). The right two frames (b) are phase-contrast photos of a torso embryo at two focal planes which also exhibits a twisted phenotype. ×210 magnification.

McKnight and Miller, 1976; Anderson and Lengyel, 1979). Histological sections of blastoderm stage embryos derived from germ-line clones of null alleles of *l(1)ph* suggest that the *l(1)ph*$^+$ gene must be expressed prior to blastoderm cellularization in the zygote. This would establish *l(1)ph* as a gene with very early activity. Other very early acting genes have recently been described, *fushi tarazu* and *zerknullt* (Wakimoto *et al.*, 1984) which exhibit a temperature-sensitive period prior to 2 hr after fertilization. Molecular analysis of the *l(1)ph*$^+$ gene, as has been accomplished for the *fushi tarazu* gene (Kuroiwa *et al.*, 1984; Hafen *et al.*, 1984), will be necessary to determine the exact time at which transcription of *l(1)ph*$^+$ begins.

Embryonic development in holometabolous insects performs two roles: it forms the larvae but in addition it produces the adult "embryo" in the form of scattered groups of cells (imaginal anlagen) which proliferate during larval development and undergo morphogenesis and differentiation during the pupal stage. It is clear that larval viability and growth are not dependent upon the presence of imaginal discs (Shearn, 1978; Szabad and Bryant, 1982). However, the imaginal tissues are, of course, required as metamorphosis begins. *l(1)ph*$^+$ is an apparent "discless" mutation. Analysis of the zygotic phenotype indicates that the *l(1)ph* gene product is required for cell proliferation of the imaginal anlagen. It is noteworthy, however, that *l(1)ph*$^+$ gene activity is not required for germ cell proliferation and differentiation in homozygous clones.

Because very few larval somatic cells divide after the early embryonic period and since normal embryonic cell division may occur under the control of maternally acting gene functions, Baker *et al.* (1982) postulated that late larval-early pupal lethal loci may represent a class of loci of which many are involved in the cell cycle. It is reasonable that some of these genes would have a maternal influence on embryonic development. The discless zygotic and the MEL embryonic phenotypes indicate that the *l(1)ph* locus appears to be involved in a basic cellular function such as epithelial cellularization or cell proliferation. We would like to suggest that many other cellular, morphological, and differentiative processes required to form the adult from the imaginal

anlagen are similarly required to form the larva from the egg. Therefore, examination of the maternal effects of genes which disrupt pupal development will be a fruitful method for identifying genes which have previously been unrecognized for their impact on embryonic development. Such genes may reveal novel maternal effect lethal phenotypes; but more importantly they may allow us to determine the maternal versus zygotic contributions to embryonic pattern determination by manipulating the relative levels of maternal and zygotic gene activities by hypomorphic versus amorphic alleles or by utilizing the temperature effects on variegating duplications of such genes.

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