DEVELOPMENTAL BIOLOGY 127, 392-407 (1988)

The Maternal Effect of *lethal(1)discs-large-1:* A Recessive Oncogene of *Drosophila melanogaster*

NORBERT PERRIMON

Howard Hughes Medical Institute and Department of Genetics, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115

Accepted February 25, 1988

The maternal effect phenotypes of recessive mutations at the *Drosophila* zygotic lethal gene *l(1)discs-large-1* (*l(1)dlg-1*) are described. *l(1)dlg-1* is located in 10B7-8 on the salivary gland chromosome map. A complex complementation pattern is observed among the nine characterized alleles. Larvae missing zygotic *l(1)dlg-1*$^+$ gene activity die due to aberrant growth of imaginal cells at the larval-pupal transition. Embryos lacking both maternal and zygotic activity of *l(1)dlg-1*$^+$, i.e., embryos derived from homozygous *l(1)dlg-1* germ line clones for null alleles, show neurogenesis and morphogenesis defects that result in very abnormal embryos. Although differentiated, most tissues are morphologically misshapen. This maternal effect is rescuable to some extent. One allele, *l(1)dlg-1*^{HF321}, is a temperature-sensitive mutation for the zygotic lethality. Embryos derived from homozygous *l(1)dlg-1*^{HF321} females at 18°C exhibit defects associated with dorsal closure and head involution. More extreme phenotypes are observed when females are shifted to higher temperatures and include defective dorsal closure, collapse of the somatic musculature, and an oversized central nervous system. The possible involvement of the recessive oncogene *l(1)dlg-1* in cell adhesion is discussed. © 1988 Academic Press, Inc.

INTRODUCTION

A large number of zygotic lethal mutations in *Drosophila* cause death at the larval-pupal transition. Most of these interfere with imaginal disc development. According to the mutant phenotypes associated with the imaginal discs, six major classes have been distinguished (Shearn, 1978), mutations which result in (1) no discs; (2) small discs due to extensive cell death; (3) small discs due to slow cell divisions and growth; (4) large discs due to overgrowth; (5) homeotic transformations; and (6) abnormal disc differentiation. The imaginal disc overgrowth mutants have been further subdivided into two groups based on the nature of the overgrowing tissue; these are (1) epithelial overgrowth mutants and (2) nonepithelial overgrowth mutants. In the first case the overgrowing mutant imaginal discs maintain their single-layered epithelial structure. In the second case the overgrowth causes breakdown of the single-layered epithelial structure thereby converting the discs into spongy masses of tissue (Bryant, 1986). Interestingly, within these spongy masses there is loss of specialized cell junctions, which is also observed in many vertebrate tumors (Gateff, 1978).

Only a few loci have been identified that when mutated lead to the formation of large tumorous discs (Gateff, 1978). It is likely that among these loci there are some which are specifically involved in the termination of cell proliferation during disc development (Bryant, 1986). Among the group of nonepithelial overgrowth mutants two loci have been extensively studied, *l(1)discs-large-1* (*l(1)dlg-1*) (Stewart *et al.*, 1972) and *l(2)giant-larvae* (*l(2)gl*) (Hadorn, 1961). Mutations at the *l(2)gl* locus have been shown to lead to malignant neoplasms after mutant imaginal discs are transplanted into wild-type female abdomens (Gateff and Schneiderman, 1967). Similarly, one allele of *l(1)dlg-1*, characterized by Gateff (1978), *l(1)benign wing imaginal disc neoplasm* (*l(1)dlg-1*^{bwn}) has been reported to generate benign neoplasms after transplantation. When recessive mutations lead to the development of neuroblastoma and imaginal disc tumors, they are referred to as recessive oncogenes or anti-oncogenes (Mechler *et al.*, 1985).

To address the specificity of the temporal expression of one of these loci during *Drosophila* development we examined the maternal effect lethal phenotype of *l(1)dlg-1* using germ line clonal analysis of both zygotic lethal mutations and a temperature-sensitive allele. Here I show that *l(1)dlg-1* has a maternal effect lethal phenotype whose product(s) is required during embryogenesis for proper neurogenesis and morphogenesis of differentiated tissue. The function of *l(1)dlg-1*$^+$ during *Drosophila* development and its possible involvement in cell adhesion are discussed.

MATERIALS AND METHODS

Strains

In Table 1 the origins of the mutations used in this study are listed. Two of the *dlg-1* alleles, *l(1)dlg-1*^{1P20} and *l(1)dlg-1*^{565}, were isolated during a screen for larval-

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TABLE 1
ORIGINS, LETHAL PHASE, AND GERM LINE CLONE ANALYSIS OF *l(1)dlg-1* ALLELES

	LP	Origin	Reference	Germ line clone analysis		
				N	NGLC	ME
l(1)dlg-1^{XI-2}	L3-P	EMS	(1)	840	21	MELR
l(1)dlg-1^{B11}	L3-P	EMS	(1)	200	10	MELR
l(1)dlg-1^{5G5}	L3-P	DEB	(2)	600	30	MELR
l(1)dlg-1^{1P20}	L3-P	EMS	(2)	350	17	MELR
l(1)dlg-1^{v55}	P	EMS	(3)	400	14	MELR
l(1)dlg-1^{RA16}	L1-2	XR	(4)	460	8	MELR
l(1)dlg-1^{M35}	L3-P	ENU	(5)	250	10	MELR
l(1)dlg-1^{M52}	L3-P	ENU	(5)	700	44	MELR
l(1)dlg-1^{HF321}	ts	XR	(4)			

Note. Origin: ethyl methanesulfonate (EMS), *N*-ethyl-*N*-nitrosourea (ENU), X-rays (XR), diepoxybutane (DEB). Lethal phase: The early lethal phase of hemizygous *l(1)dlg-1*^{RA16} progeny is attributable to a second site lethal mutation(s) since males of genotype *l(1)dlg-1*^{RA16}/*Dp(1;Y)*v^+Yy^+ are not recovered and females of genotype *l(1)dlg-1*^{RA16}/*Df(1)N71* die at a later stage (L3-P) than *l(1)dlg-1*^{RA16}/*Y* males. Most *l(1)dlg-1*^{v55} hemizygous progeny derived from heterozygous mothers pupate. This is due to the hypomorphic nature of the mutation (see text). The lethal phase of each mutation is indicated (L1, L2, L3: larval stages; P: pupal stage). One allele, *l(1)dlg-1*^{HF321} is a temperature-sensitive (ts) lethal (see text). References: (1) D. Woods and P. Bryant, unpublished results; (2) this study; (3) Geer *et al.* 1983; (4) G. Lefevre, unpublished results; (5) R. Voelker, unpublished results. Germ line clone analyses: Maternal expression (ME) was determined by germ line clone analysis. *N* is the number of females of genotype *Fs(1)K1237*v^{24}/*l(1)dlg-1* analyzed and NGLC corresponds to the number of females possessing a germ line clone. MELR refers to a paternally rescuable maternal effect lethal phenotype.

pupal lethal mutations on the X chromosome (N. Perrimon, unpublished results), while the others were obtained from various sources (see Table 1). We also utilized a deficiency, *Df(1)N71* (Df(1)10B5-D4), and a duplication, *Dp(1;Y)*v^+Yy^+ *Dp(1;Y)* (9F3 to 10C1-2; 20B to base; 1A1 to 1B2; *Y*), of the *dlg-1* locus (Craymer and Roy, 1980). for complementation tests. Descriptions of stocks and balancers, unless identified in the text, can be found in Lindsley and Grell (1968). Experiments were conducted at 18, 20, 25, or 29°C on standard *Drosophila* medium.

Germ Line Clone Analysis

Germ line clones were induced using the dominant female sterile technique (Perrimon and Gans, 1983; Perrimon *et al.*, 1984a). It utilizes the X-linked germ line-dependent dominant female sterile mutation *Fs(1)K1237* (or *ovo*^{D1}, Busson *et al.*, 1983; Perrimon, 1984) which is maintained in an attached-X stock: *C(1)DX,yf/Y* females crossed to *Fs(1)K1237*v^{24}/*Y* males. To induce germ line clones homozygous for the *l(1)dlg-1* mutation the progeny of *Balancer/l(1)dlg-1* females mated to males carrying the dominant female sterile mutation *Fs(1)K1237* were irradiated at the end of the first instar larval stage. A constant dose of 1000 rad was used (Torrex 120D X-ray machine; 100Kv, 5 mA, 3-mm aluminum filter). At emergence, irradiated *l(1)dlg-1/Fs(1)K1237* females were examined for the presence of eggs, then isolated, and studied individually. Such experimental conditions generate 5 to 8% mosaic females on the X chromosome.

Temperature Shift Experiments of l(1)dlg-1^{HF321}

To determine the temperature-sensitive period of *l(1)dlg-1*^{HF321}, eggs from the cross *FM3/l(1)dlg-1*^{HF321} by *l(1)dlg-1*^{HF321}/*Dp(1;Y)*v^+Yy^+ were collected for a period of 12 hr at 18°C. These eggs were allowed to develop either at the permissive (18°C) or restrictive (25 or 29°C) temperature until the up or downshift was performed. The percentage of viability of the homozygous mutant progeny is calculated as the number of *l(1)dlg-1*^{HF321}/*l(1)dlg-1*^{HF321} adult females divided by the total number of *FM3/l(1)dlg-1*^{HF321} adult females. In each cross at least 100 *FM3/l(1)dlg-1*^{HF321} females were scored.

Analysis of Zygotic Phenotypes

Embryos were examined by three methods: (1) embryonic cuticles were prepared according to the Hoyer's mount technique of Van der Meer (1977); (2) histological sections were prepared as described by Mahowald *et al.* (1979); and (3) scanning electron micrographs were prepared as described by Turner and Mahowald (1976). The vitelline membranes were removed according to the technique of Mitchison and Sedat (1983) and modified by Dequin *et al.* (1984).

Lethal phase determination was performed as described by Perrimon *et al.* (1984a).

RESULTS

l(1)discs-large-1 Mutations

l(1)discs-large-1 (*l(1)dlg-1*) maps on the X chromosome at meiotic position 34.82 (Stewart *et al.*, 1972; Geer *et al.*, 1983). Cytologically the locus maps in bands 10B7-8 of the salivary gland chromosomes. The locus is defined by the distal breakpoints of *Df(1)DA622* and *Df(1)m13* where it is included within *Df(1)DA622* and excluded from *Df(1)m13* (Perrimon and Mahowald, 1986; Bryant, 1986).

We have analyzed in detail nine alleles of the *l(1)discs-large-1* (*dlg-1*) locus (Table 1). Eight of the alleles ($l(1)dlg\text{-}1^{M52}$, $l(1)dlg\text{-}1^{M35}$, $l(1)dlg\text{-}1^{RA16}$, $l(1)dlg\text{-}1^{XI\text{-}2}$, $l(1)dlg\text{-}1^{l11}$, $l(1)dlg\text{-}1^{v55}$, $l(1)dlg\text{-}1^{565}$ and $l(1)dlg\text{-}1^{1P20}$) behave as zygotic lethals and never result in hemi- or homozygous adults at 18, 25, or 29°C. Only one temperature-sensitive allele, $l(1)dlg\text{-}1^{HF321}$, can result in some hemi- and homozygous adults at the permissive temperature (18°C).

For six *l(1)dlg-1* alleles ($l(1)dlg\text{-}1^{M52}$, $l(1)dlg\text{-}1^{M35}$, $l(1)dlg\text{-}1^{XI\text{-}2}$, $l(1)dlg\text{-}1^{l11}$, $l(1)dlg\text{-}1^{565}$, and $l(1)dlg\text{-}1^{1P20}$) the lethal phase of hemizygous male progeny derived from heterozygous females occurs during larval-pupal stages (Table 1). The phenotype of the hemizygous males is indistinguishable from the phenotype of hemizygous females which are heterozygous for anyone of these mutations and a deficiency of the region at both 18 and 25°C. The stage of lethality of the hemizygous progeny is variable, where 6 days after oviposition only 10% of the mutant larvae have pupated and after 10 days approximately 60% have pupated. The developmental stage reached by the pupae is variable and in some cases very little development is observed. The remaining 30% mutant larvae will never enter pupariation and will eventually die after an extended larval period that can last up to 2 weeks. During the extended larval period mutant imaginal discs continue to grow beyond their normal limits and become irregular in shape. These discs have been referred to as spongy masses of tissue (Bryant, 1986).

The remaining two zygotic lethal mutations, $l(1)dlg\text{-}1^{RA16}$ and $l(1)dlg\text{-}1^{v55}$, differ from the six described above. $l(1)dlg\text{-}1^{v55}$ is a temperature-sensitive hypomorphic allele whose lethal phase in homozygous progeny occurs mainly during pupal stages and is shifted to an earlier stage in $Df(1)N71/l(1)dlg\text{-}1^{v55}$ individuals. $l(1)dlg\text{-}1^{RA16}/Y$ larvae die during early larval stages due to the presence of a second site lethal mutation(s) (see Table 1).

$l(1)dlg\text{-}1^{HF321}$ clearly behaves as a temperature-sensitive allele. We analyzed the progeny from a cross between $FM3/l(1)dlg\text{-}1^{HF321}$ females and $l(1)dlg\text{-}1^{HF321}/Dp(1;Y)v^{+}Yy^{+}$ males. No homozygous $l(1)dlg\text{-}1^{HF321}$ adult flies are obtained at either 25 and 29°C. However, when compared to the heterozygous siblings at 18°C, the viability of the homozygous females is 37% (Table 2). The viability of hemizygous $l(1)dlg\text{-}1^{HF321}/Y$ males obtained from a cross between $FM3/l(1)dlg\text{-}1^{HF321}$ females and $+/Y$ males is 50%. Homozygous $l(1)dlg\text{-}1^{HF321}$ females are poorly fertile at 18°C and fully sterile above 21°C (see below and Table 3), but hemizygous males are fertile at all temperatures.

Temperature shifts were performed to determine the temperature-sensitive period of the zygotic lethality (see Materials and Methods). When progeny from a cross between $FM3/l(1)dlg\text{-}1^{HF321}$ females and $l(1)dlg\text{-}1^{HF321}/Dp(1;Y)v^{+}Yy^{+}$ males are shifted from the permissive (18°C) to the restrictive temperature (25 or

TABLE 2
TRANS-ALLELIC COMPLEMENTATION BETWEEN *l(1)dlg-1* ALLELES

	T (°C)	*N71* (%)	*X1-2* (%)	*l11* (%)	*M35* (%)	*1P20* (%)	*v55* (%)	*HF321* (%)
X1-2	25	0	0					
l11	25	0	0	0				
M35	25	0	0	0	0			
1P20	25	0	0	0	95	0		
v55	18	4	2	0	10	0	0	
	25	0	0	0	0	0	0	
HF321	18	68	65	0	0	98	91	37
	25	0	0	0	0	95	4	0

Note. Crosses between two *l(1)dlg-1* alleles (refered to as $l(1)dlg\text{-}1^{a}$ and $l(1)dlg\text{-}1^{b}$) were performed by crossing $FM3/l(1)dlg\text{-}1^{a}$ females to $l(1)dlg\text{-}1^{b}/Dp(1;Y)v^{+}Yy^{+}$ males. Crosses were performed at two different temperatures (T = 18 or 25°C) and in two different ways: (1) $l(1)dlg\text{-}1^{a}$ was introduced by the female parent, or (2) $l(1)dlg\text{-}1^{a}$ was introduced by the male parent (except in the case of $l(1)dlg\text{-}1^{RA16}$ where no males of genotype $l(1)dlg\text{-}1^{RA16}/Dp(1;Y)v^{+}Yy^{+}$ could be recovered). No differences were observed whether the allele was introduced from the female or the male. In complementation tests $l(1)dlg\text{-}1^{RA16}$, $l(1)dlg\text{-}1^{565}$ and $l(1)dlg\text{-}1^{M52}$ behave as $l(1)dlg\text{-}1^{l11}$ (results not shown). *N71* refers to *Df(1)N71* a deficiency of the *dlg-1* locus (see Materials and Methods). The percentage of viability is calculated as follows: % = 100 times the number of $l(1)dlg\text{-}1^{a}/l(1)dlg\text{-}1^{b}$ females divided by the number of $FM3/l(1)dlg\text{-}1^{a}$ females (in the cases where $l(1)dlg\text{-}1^{a}$ was introduced from the female parent). In each cross at least 150 $FM3/l(1)dlg\text{-}1^{a}$ females were scored.

TABLE 3
FERTILITY OF $l(1)dlg\text{-}1^{HF321}$

T (°C)	N days	Hatchability			Embryonic phenotypes				
		N	N_{unf}	%H	WT	1	2	3	4
		HF321/HF321 × HF321/Y							
18	5	425	60	4	4	86	10	0	0
	10	625	102	3	1	84	15	0	0
25	1	325	15	0	2	10	83	5	0
	3	410	42	0	3	5	80	12	0
	10	400	50	0	2	7	19	72	0
29	1	250	10	0	2	3	17	76	2
	3	300	60	0	1	5	4	6	84
	10	350	314	0	3	2	2	3	90
		HF321/HF321 × +/Y							
18	5	352	28	47					
	10	410	50	45					
25	2	310	10	52					
	4	360	26	45					
	10	250	80	48					
29	2	300	10	49					
	4	400	50	44					
	10	300	250	30					

Note. Homozygous $l(1)dlg\text{-}1^{HF321}$ females were obtained by crossing $FM3/l(1)dlg\text{-}1^{HF321}$ females with $l(1)dlg\text{-}1^{HF321}/Y$ males at 18°C. These homozygous $l(1)dlg\text{-}1^{HF321}$ females were crossed to either $l(1)dlg\text{-}1^{HF321}/Y$ males or wild-type *Oregon R* males (+/Y). Following the cross these females were immediately subdivided into three groups (18, 25, and 29°C) and their fertility was analyzed at various times (N days refers to the number of days following the temperature shift at which the fertility was analyzed). The test for fertility was as follows: eggs were collected over a period of 12 hr and lined up on a fresh media, after 24 hr the number of hatched larvae was counted, and the unhatched eggs were examined after cuticle preparation. Fertility was assayed as the percentage of hatched embryos: % hatching (%H) is 100 times the number of larvae divided by N_f (N_f = number of eggs (N) − number of unfertilized eggs (N_{unf})). The phenotype of the unhatched embryos has been subdivided into five phenotypic classes: wild-type (WT) and classes 1 to 4 (see text and the legend to Fig. 1).

29°C) during embryonic, larval, and early pupal stages no homozygous progeny are obtained. However, homozygous females are recovered if the upshift occurs during late pupal development and the frequency of recovery increases as the shift comes closer to the time of emergence. For example, if the shift (from 18 to 29°C) is performed 3 days before emergence no homozygous females are recovered. However, a shift done 2 days before emergence results in 7% homozygous flies and a shift done 1 day before emergence results in 15% homozygous flies. Similar results were obtained with shift from 18 to 25°C.

Downshift experiments, from the restrictive (25 or 29°C) to the permissive temperature (18°C), indicate that it is possible to recover homozygous $l(1)dlg\text{-}1^{HF321}$ females as late as the early third instar stage. No homozygous progeny are obtained if the downshift is done after the third larval stage. However, homozygous females are recovered if the downshift occurs earlier during larval development and the frequency of recovery increases if the shift is done earlier. For example, if the shift (from 29 to 18°C) is performed during late third instar larval stage no homozygous females are recovered. However, a shift done during early third instar larval stage results in 2% homozygous mutant flies. A shift done during second instar larval stage results in 8% homozygous flies and a shift done during the first instar larval stage results in 24% homozygous flies. Similar results were obtained with downshift from 25 to 18°C. Interestingly, about 10% of the homozygous mutant adults derived from larvae shifted during the second instar larval stage exhibit somatic defects, most of which are unilateral and involve eye development.

These temperature shift experiments demonstrate that the $l(1)dlg\text{-}1^{+}$ gene product is required during both larval and pupal stages.

l(1)dlg-1 Is a Complex Locus

Complementation analyses between *discs-large-1* alleles indicate that the locus is genetically complex. Based on the complementation pattern shown in Table 2 four groups of mutations can be distinguished.

Group 1: Null allele ($l(1)dlg\text{-}1^{XI\text{-}2}$). In complementation tests this allele behaves as a deficiency of the locus; like *Df(1)N71* (see Materials and Methods). No complementation is observed with any of the group 2 or group 3 *dlg-1* mutations. However, to some extent, complementation is observed with the class 4 hypomorphic alleles $l(1)dlg\text{-}1^{HF321}$ and $l(1)dlg\text{-}1^{v55}$ (Table 2).

Group 2: Alleles that show negative complementation ($l(1)dlg\text{-}1^{M52}$, $l(1)dlg\text{-}1^{RA16}$, $l(1)dlg\text{-}1^{l11}$, and $l(1)dlg\text{-}1^{565}$). We have characterized four alleles within this group and find no complementation with any *l(1)dlg-1* alleles. It is particularly surprising that no complementation is observed with $l(1)dlg\text{-}1^{HF321}$ at 18°C since some $Df(1)N71/l(1)dlg\text{-}1^{HF321}$ females are recovered at 18°C. This result indicates that these mutations are not null alleles of the gene since they do not behave as a deficiency of the locus. Interaction of this kind has been termed negative complementation (see Discussion).

Group 3: Alleles that show interallelic complementation ($l(1)dlg\text{-}1^{M35}$ and $l(1)dlg\text{-}1^{1P20}$). $l(1)dlg\text{-}1^{1P20}$ fully complements the zygotic lethality of $l(1)dlg\text{-}1^{M35}$ and $l(1)dlg\text{-}1^{HF321}$ both at 18 and 25°C. $l(1)dlg\text{-}1^{M35}$ also complements to some extent $l(1)dlg\text{-}1^{v55}$ at 18°C.

Group 4: Temperature-sensitive alleles ($l(1)dlg\text{-}1^{v55}$ and $l(1)dlg\text{-}1^{HF321}$). $l(1)dlg\text{-}1^{HF321}$ behaves as a temperature-sensitive hypomorphic allele (see above). Over a deficiency and group 1 mutations partial complementation (up to 65%) is observed. Interestingly, females trans-heterozygous with group 1 mutations and *Df(1)N71* have better viability than females homozygous for $l(1)dlg\text{-}1^{HF321}$. This discrepancy can be attributed to the homozygosity of the $l(1)dlg\text{-}1^{HF321}$ chromosome. $l(1)dlg\text{-}1^{v55}$ hemi- and homozygous flies are never recovered at 18, 25, or 29°C. However, over a deficiency and the group 1 allele some trans-heterozygous flies are recovered at a low frequency (less than 5%). Homozygosity of the $l(1)dlg\text{-}1^{v55}$ chromosome may be responsible for such an effect. This complementation pattern suggests that $l(1)dlg\text{-}1^{v55}$ is a weak temperature-sensitive allele. This is in agreement with the complementation pattern observed between $l(1)dlg\text{-}1^{v55}$ and $l(1)dlg\text{-}1^{HF321}$ where at 18°C trans-heterozygous flies have very good viability (91%) but the recovery of these flies at 25°C is less than 5%.

The dlg-1 Embryonic Phenotypic Series

Embryos derived from germ line clones homozygous for zygotic lethal alleles of *l(1)dlg-1* as well as embryos derived from homozygous $l(1)dlg\text{-}1^{HF321}$ females exhibit a variety of embryonic phenotypes. All of these phenotypes constitute a phenotypic series (classes 1 through 5, Fig. 1) in which the severity of the embryonic phenotype depends upon the level of $l(1)dlg\text{-}1^{+}$ gene activity, where the more reduced the level of $l(1)dlg\text{-}1^{+}$ gene activity the more severe the embryonic phenotype. These five major phenotypic classes have been distinguished according to the uniqueness of the embryonic defects observed. For a description of wild-type development see Turner and Mahowald (1976, 1977) and Campos-Ortega and Hartenstein (1985).

Class 1 embryos (Figs. 1B1 and 1B2). This class exhibits localized defects in dorsal closure at the most dorsal and posterior part of the embryo. This phenotype is only observed in eggs derived from homozygous $l(1)dlg\text{-}1^{HF321}$ females at 18°C.

Class 2 embryos (Figs. 1C1 and 1C2, 2A–2C, and 3B). The dorsal closure defect extends to the entire posterior third of the embryo (Figs. 1C1 and 1C2). Additionally, the head does not involute, the brain protrudes anteriorly, and most amnioserosa cells degenerate in the region where dorsal closure is defective. All internal structures, i.e., central nervous system, musculature, and gut, are well differentiated (Figs. 2A–2C and 3B). Since the dorsal epidermis does not close, large holes are present in the differentiated cuticle; however, the differentiation of the ventral epidermis, visualized by the pattern of denticle belts, is not affected (Figs. 1C1 and 1C2).

Class 3 embryos (Figs. 1D; 2D, 2E, 3C–3E, and 4b–4E). The head and dorsal side of the embryo is fully open. These embryos are missing head and dorsal cuticle, but possess two well-developed posterior spiracles with filzkorper material. Additionally, the pattern of ventral denticles is normal. Some residual parts of the cephalopharyngeal apparatus are present.

The first defects associated with these embryos can be detected after germ band shortening, at the time of dorsal closure at 10 hr of embryonic development. The dorsal epidermis does not fuse at the dorsal midline, while cells from the amnioserosa become "loose" and eventually degenerate. In most embryos the midgut closes dorsally. At 12 hr of development (Figs. 4B–4D) the dorsal epidermis is situated laterally, most amnioserosa cells have disappeared; the supraesophageal ganglia, midgut, and hindgut protrude dorsally; and head involution has not occurred. Amnioserosa cells very likely degenerate (see cell death in Fig. 4D).

The lateral somatic musculature (i.e., the pleural external transverse muscles (Campos-Ortega and Hartenstein, 1985)), although well differentiated, do not attach or have lost their connections to the lateral apodemes, and finally "collapse" ventrally. The ventral somatic musculature (i.e., ventral internal oblique and ventral superficial oblique muscles) are well differentiated and attached to their appropriate apodemes. Figure 4E shows a section through an embryo at 17 hr of development where the collapse of the lateral musculature is evident.

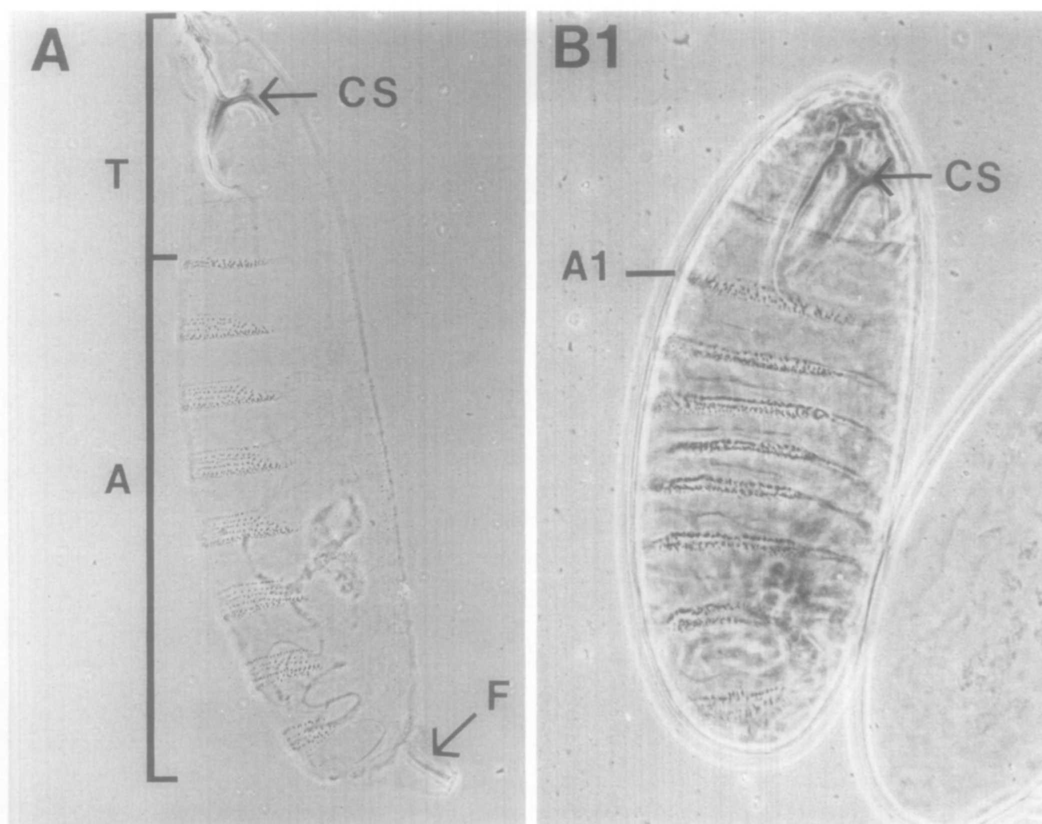

FIG. 1. Cuticle phenotype of *discs-large-1* embryos. (A) A phase-contrast micrograph of a wild-type embryo. Note the three thoracic (T) and eight abdominal (A) segments as well as the filzkorper (F) and the cephalopharyngeal skeleton (CS). Embryos shown in (B) through (F) are of genotype $l(1)dlg\text{-}1^{HF321}/Y$. They are derived from $l(1)dlg\text{-}1^{HF321}/l(1)dlg\text{-}1^{HF321}$ females crossed to wild-type males (+/*Y*) at various temperatures (see text and Table 3). (B1, B2) Two different focal planes of a class 1 embryo. The open arrow in (B2) indicates the dorsal closure defect. (C1, C2) Two different focal planes of a class 2 embryo. Note the normal segmental pattern of denticle belts in C1 and the opening of the head and posterior regions on the dorsal side of the embryo in C2 (indicated by the stars). The filzkorper are detectable in this embryo. (D) A dorsal view of a class 3 embryo where the dorsal side is completely open. The dorsal epidermis has contracted laterally (indicated by the open arrows). (E1, E2) Two different focal planes of a class 4 embryo. Note the degeneration of both the dorsal and lateral epidermis (open arrow in E2) and the ventral epidermis (star in E1). (F) An extreme class 4 embryo where cuticle structure is grossly abnormal. (G) A class 5 embryo of genotype $l(1)dlg\text{-}1^{M52}/Y$ which is derived from a germ line clone homozygous for the $l(1)dlg\text{-}1^{M52}$ allele. This embryo has not received a paternal copy of $l(1)dlg\text{-}1^{+}$. No cuticle pattern is detectable.

At 12 hr of development the central nervous system appears normal, with developed commissures, longitudinals, and neuropile. After 12 hr of development the central nervous system becomes misshapen and enlarges dramatically in size (compare Figs. 3E and 3A). After 18 hr of development extensive cell death is detectable within the central nervous system.

In late embryogenesis the epidermis, though cellularly normal, becomes condensed ventrally probably as a result of the contraction of the ventral musculature. Also, the dorsal protrusion of internal structures becomes accentuated (Figs. 2D and 2E). This is evident in Fig. 2E, a scanning electron micrograph of an embryo at 19 hr of development, and in Figs. 3D and 3E, sections of slightly younger embryos.

Class 4 embryos (Figs. 1E1, 1E2, 1F, and 3F). These embryos are like class 3 embryos except more severe, where all the dorsal side is open. The unique feature of this class is that ventral epidermis structures are affected. The lateral epidermis is necrotic (Fig. 1E2) and large patches of ventral cuticle are missing (Fig. 1E1). Nervous tissue from the ventral nerve cord protrudes through the holes left by the missing ventral epidermis. At 16 hr of development (Fig. 3F), the embryo is composed of well-differentiated tissues that are abnormally shaped; the supraesophageal ganglia, midgut, and hindgut protrude dorsally; the salivary glands are misshapen and protrude ventrally; all somatic musculature is aberrant both in shape and position; the ventral epidermis is falling apart; and the ventral nerve cord protrudes ventrally. The extensive degeneration of the epidermis prevents most cuticle secretion, which is responsible for the poorly differentiated appearance of the embryos when analyzed in cuticular mounts (Fig. 1F).

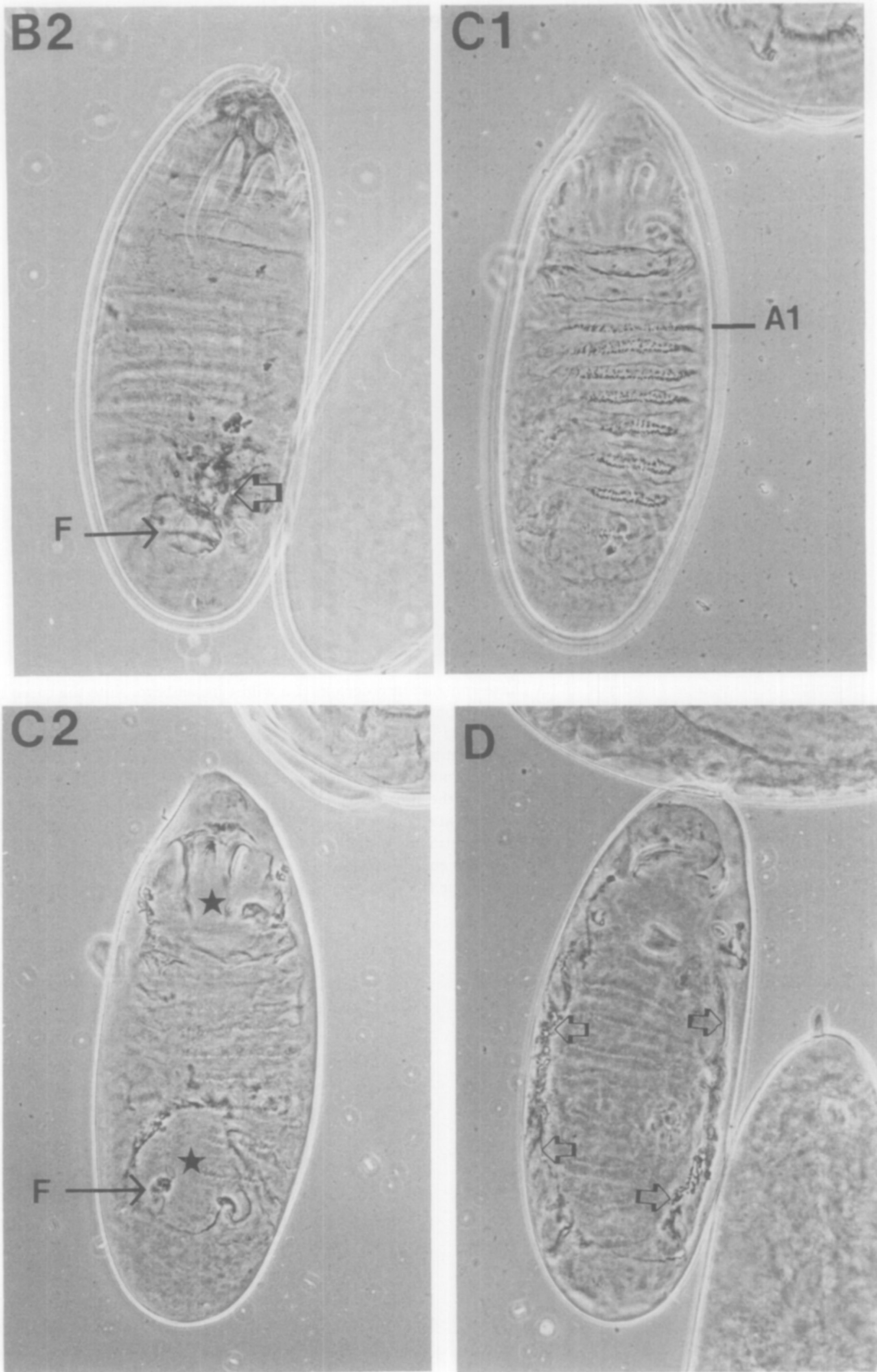

FIG. 1.—*Continued.*

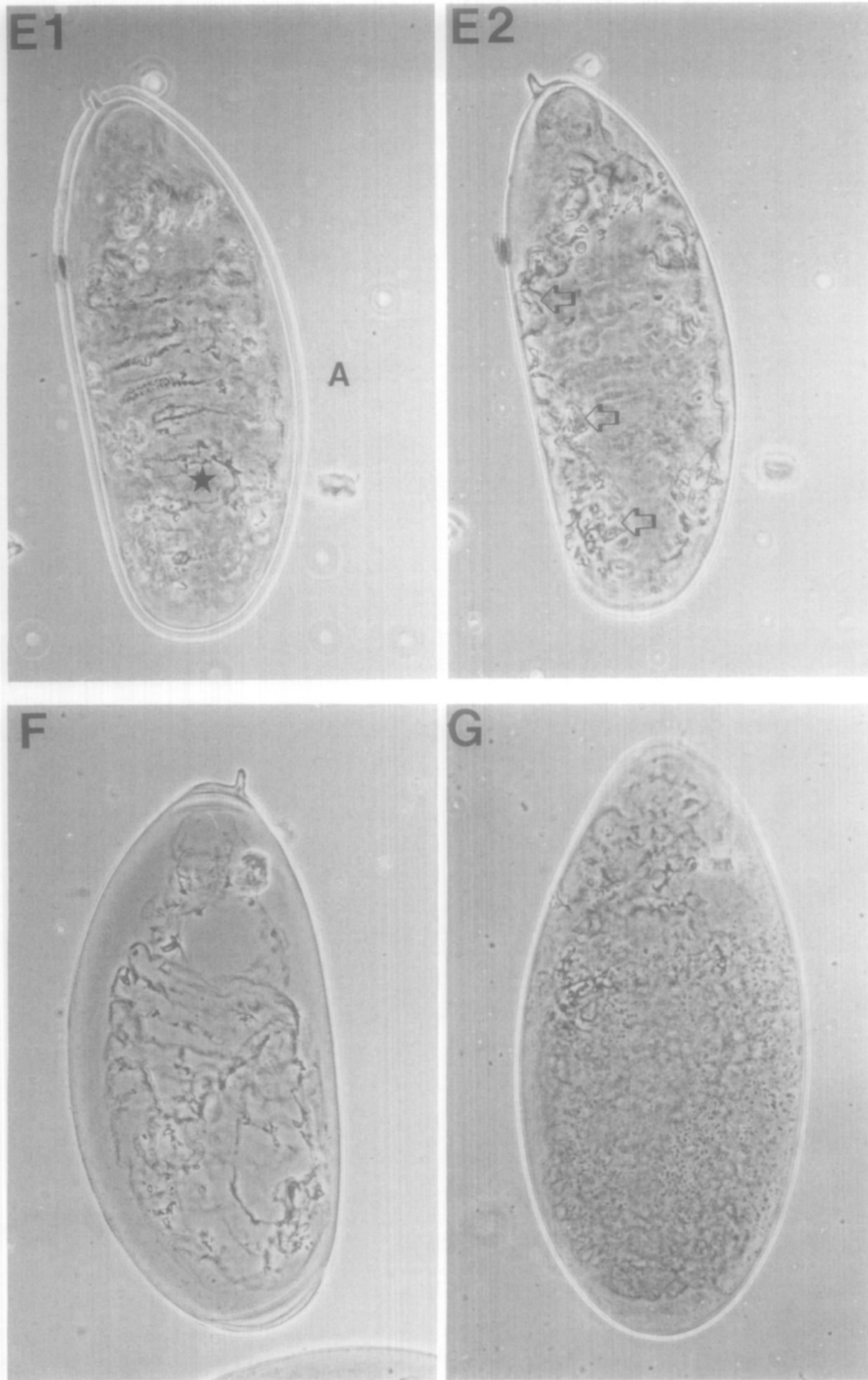

FIG. 1.—*Continued.*

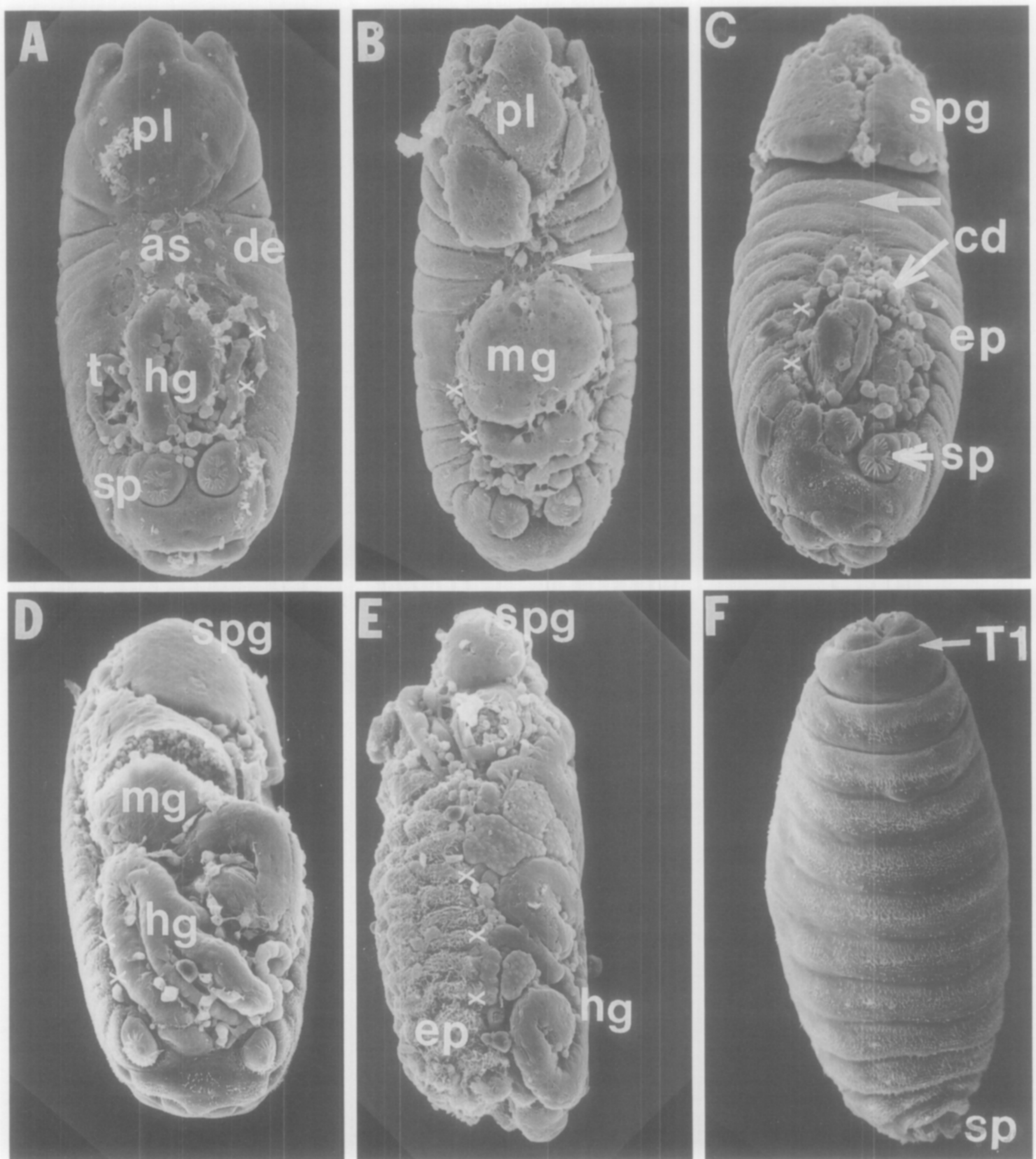

FIG. 2. Dorsal closure defect in *l(1)dlg-1*^{HF321} embryos visualized by scanning electron micrographs. Embryos depicted in (A–E) are derived from homozygous *l(1)dlg-1*^{HF321} females crossed to *l(1)dlg-1*^{HF321}/*Y* males at 20°C. A variety of defects in dorsal closure are observed. (A–C) Class 2 embryos at 11 to 12 hr of development. (A) An embryo at the end of germ band shortening; dorsal closure does not seem to proceed correctly and results in the dorsal appearance of gut structures. (B, C) Slightly older embryos. The arrow in (B) shows that dorsal closure has occurred only in the thoracic segments. (C) A weaker phenotype where dorsal closure is more complete. (D, E) Class 3 embryos at 15 and 19 hr of development, respectively. In (D) all of the dorsal side of the embryo is open and the midgut, brain, and gut are clearly apparent at the dorsal surface. In (E) the cuticle is well-differentiated. (F) A wild-type embryo at 19 hr of development. Note: In all Figures the white crosses refer to the boundary between the dorsal epidermis and other somatic tissue. Dorsal is up in (A–D and F). Dorsal is right in (E). Anterior is up in all figures. Nomenclature: amnioserosa, as; cell death, cd; dorsal epidermis, de; epidermis, ep; first thoracic segment, T1; hindgut, hg; midgut, mg; procephalic lobe, pl; spiracle, sp; supraesophageal ganglia, spg; trachea, t.

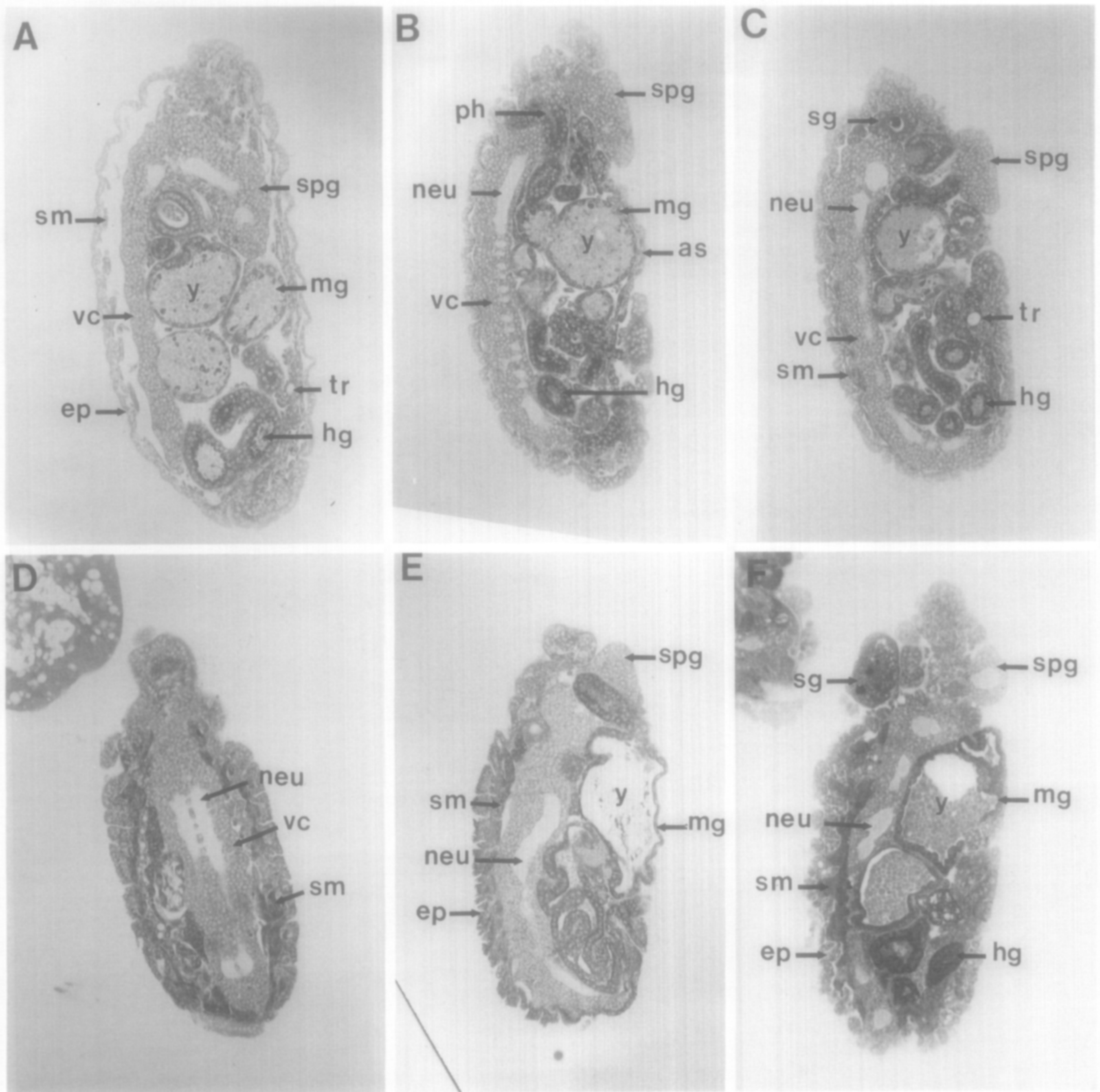

FIG. 3. Embryonic sections of *dlg-1* embryos. (A) A parasagittal section of a wild-type embryo at 17 hr of development. Note the size of the nervous system, the well-differentiated gut structures, and the presence of the somatic musculature. For the genotype of the mutant embryos see the legend to Fig. 1. (B) A parasagittal section of a class 2 embryo at 13 hr of development. The supraesophageal ganglia protrude dorsally and remnants of amnioserosa cells are present. The neuropile of the ventral nerve cord appears normal. (C–E) The phenotypes of class 3 embryos, at 14, 15, and 16 hr of embryonic development, respectively. (C, D) Parasagittal sections; (D) Frontal section. Note the presence in (C) and (E) of very abnormal somatic musculature. The embryo in (E) has an enormous nervous system, while the supraoesophageal ganglia, midgut, and hindgut protrude dorsally, and all amnioserosa tissue is missing. (F) A parasagittal section of an extreme class 4 embryo at 16 hr of development. Note the pronounced abnormalities of the salivary gland, central nervous system, and somatic musculature. Additionally, the ventral epidermis is loose and seems to degenerate. Nomenclature: amnioserosa, as; epidermis, ep; hindgut, hg; midgut, mg; neuropile, neu; pharynx, ph; salivary gland, sg; somatic musculature, sm; supraesophageal ganglia, spg; trachea, t; ventral cord, vc; yolk, y.

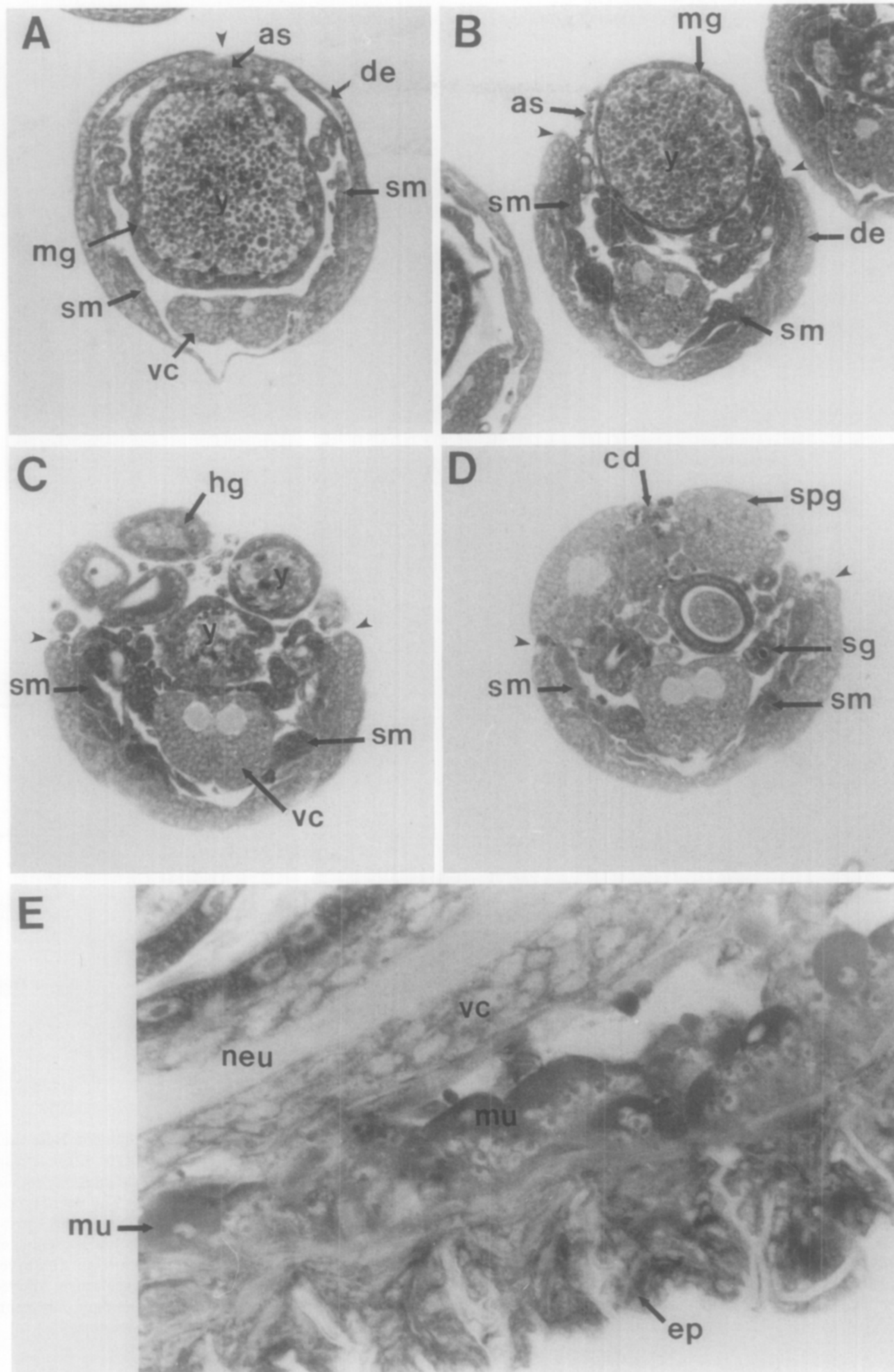

FIG. 4. Dorsal closure defects. (A–D) Transverse sections of wild-type (A) and class 3 mutant embryos (B–D) at 12 hr of development. These mutant embryos are of genotype *l(1)dlg-1*^{HF321}/*Y*. They are derived from a cross between *l(1)dlg-1*^{HF321}/*l(1)dlg-1*^{HF321} females to wild-type males (+/*Y*). They illustrate the dorsal closure defects of the epidermis. (E) A high-magnification micrograph that shows the collapsed phenotype of the lateral musculature in a 17-hr-old embryo. However, differentiated muscle cells are abnormally shaped most likely because they have not formed connections to their lateral apodemes. Nomenclature: muscle cells, mu; cell death, cd; and see legend to Fig. 3.

Class 5 embryos (Figs. 1G, 5B, and 5C). We examined embryonic sections of these poorly differentiated embryos to determine their phenotypes. Eggs from homozygous germ line clones were collected and sectioned at various stages of embryonic development. In all embryos a correct blastoderm is formed and gastrulation begins normally. The first noticeable defects are associated with neurogenesis.

In wild-type embryos at about 4 hr after fertilization following mesoderm invagination, neuroblasts segregate internally from the neuroectoderm region (Fig. 5A). In the ventral cord neuroblast segregation occurs in three waves (Campos-Ortega and Hartenstein, 1985). These neuroblasts following segregation begin to divide asymmetrically in a stem cell fashion, with each division producing a larger neuroblast and a smaller ganglion mother cell which itself only divides once synchronously to produce two daughter neurons. These neurons differentiate and in doing so extend their axons.

In mutant embryos at 4 hr of development two layers of large cells that ressemble neuroblasts are observed. One is located in the correct position, between the mesoderm and ectoderm sheaths, and the second is located at the surface of the ectodermal layer (Figs. 5B and 5C). Cell divisions are frequently observed in this layer. These embryos frequently twist during germ band elongation. Pronounced morphogenesis defects, more extreme than those observed in class 4 embryos, are subsequently observed in these class 5 embryos. Finally, extensive degeneration of the epiderm prevents cuticle secretion (Fig. 1G).

Female Sterility Phenotype of l(1)dlg-1^{HF321}

Females homozygous for the hypomorphic allele *l(1)dlg-1*^{HF321} can be obtained at 18°C (see above). Embryos hemi- or homozygous for *l(1)dlg-1*^{HF321} derived from such females exhibit a variety of embryonic phenotypes (classes 1 through 4, Fig. 1). The zygotic level of *l(1)dlg-1*$^{+}$ gene activity can be modified by temperature shift and the embryonic defects observed form a phenotypic series clearly showing progressively severe phenotypes. With progressively longer time at high temperature the extent of the embryonic defects become more extreme (Table 3).

At 18°C homozygous *l(1)dlg-1*^{HF321} females are poorly fertile when crossed to *l(1)dlg-1*^{HF321}/*Y* males; approximatively 4% of the embryos hatch (Table 3). This poor fertility does not vary with the age of the females. When homozygous *l(1)dlg-1*^{HF321} females, grown at 18°C and crossed to *l(1)dlg-1*^{HF321}/*Y* males, are shifted to 25 or 29°C, they are fully sterile. However, if females of the same genotype have been mated to wild-type males, a fraction of the embryos, corresponding to those that have received the wild-type copy of the *l(1)dlg-1*$^{+}$ gene, hatch and result in viable and fertile female progeny (Table 3). The extent of the paternal zygotic rescue is almost fully penetrant at both 18 and 25°C but decreases at 29°C. The female sterility phenotype of *l(1)dlg-1*^{HF321} can be attributable solely to a germ line defect since similar results are obtained from both germ line clones and homozygous females (results not shown). These results indicate that the genotype of the somatic tissue does not influence the maternal effect lethal phenotype.

The Maternal Effect of Amorphic l(1)dlg-1 Alleles

To address the role of the eight larval–pupal lethal alleles of *l(1)dlg-1* during oogenesis we utilized the dominant female sterile technique (Perrimon *et al.*, 1984a, see Materials and Methods for experimental details) to prepare germ line clones. All of them show similar results (Table 1); therefore, we only provide the results from one allele, *l(1)dlg-1*^{M52} (Table 4). Females possessing homozygous germ line clones when crossed to wild-type males produce three classes of phenotypically different embryos. One-half of the embryos exhibit poor cuticular differentiation (Class 5 embryos, Fig. 1G). One fourth of the embryos exhibit a cuticle pattern resembling the head and dorsal open phenotype previously observed in homozygous *l(1)dlg-1*^{HF321} females raised at 18°C (Class 1 and 2 embryos, Figs. 1B1, 1B2, 1C1, and 1C2). The remaining embryos hatch and produce female progeny. Interestingly, rescued adult female progeny derived from homozygous germ line clones of *l(1)dlg-1*^{l11}, *l(1)dlg-1*^{M35}, and *l(1)dlg-1*^{M52} exhibit a high frequency of somatic defects. About 10% of these rescued adult progeny have abdomens with partial deletions of some tergites (data not shown). These defects were not observed from homozygous germ line clones of *l(1)dlg-1*^{v55}, *l(1)dlg-1*$^{XI\text{-}2}$, and *l(1)dlg-1*^{RA16}.

To unambiguously address the genotype of the poorly differentiated embryos (or class 5 embryos), females possessing germ line clones were crossed to males carrying a wild-type copy of *l(1)dlg-1*$^{+}$ both on the X and the Y chromosomes (male genotype is $+/Dp(1;Y)v^{+}Yy^{+}$). No poorly differentiated embryos were observed. Instead, only two classes of embryos were found, embryos that develop normally and lead to viable adults and embryos with dorsal closure and head involution defects. These results indicate that lack of both maternal and zygotic functions of *l(1)dlg-1*$^{+}$ results in embryos with poor cuticle differentiation. This maternal effect is rescuable to some extent.

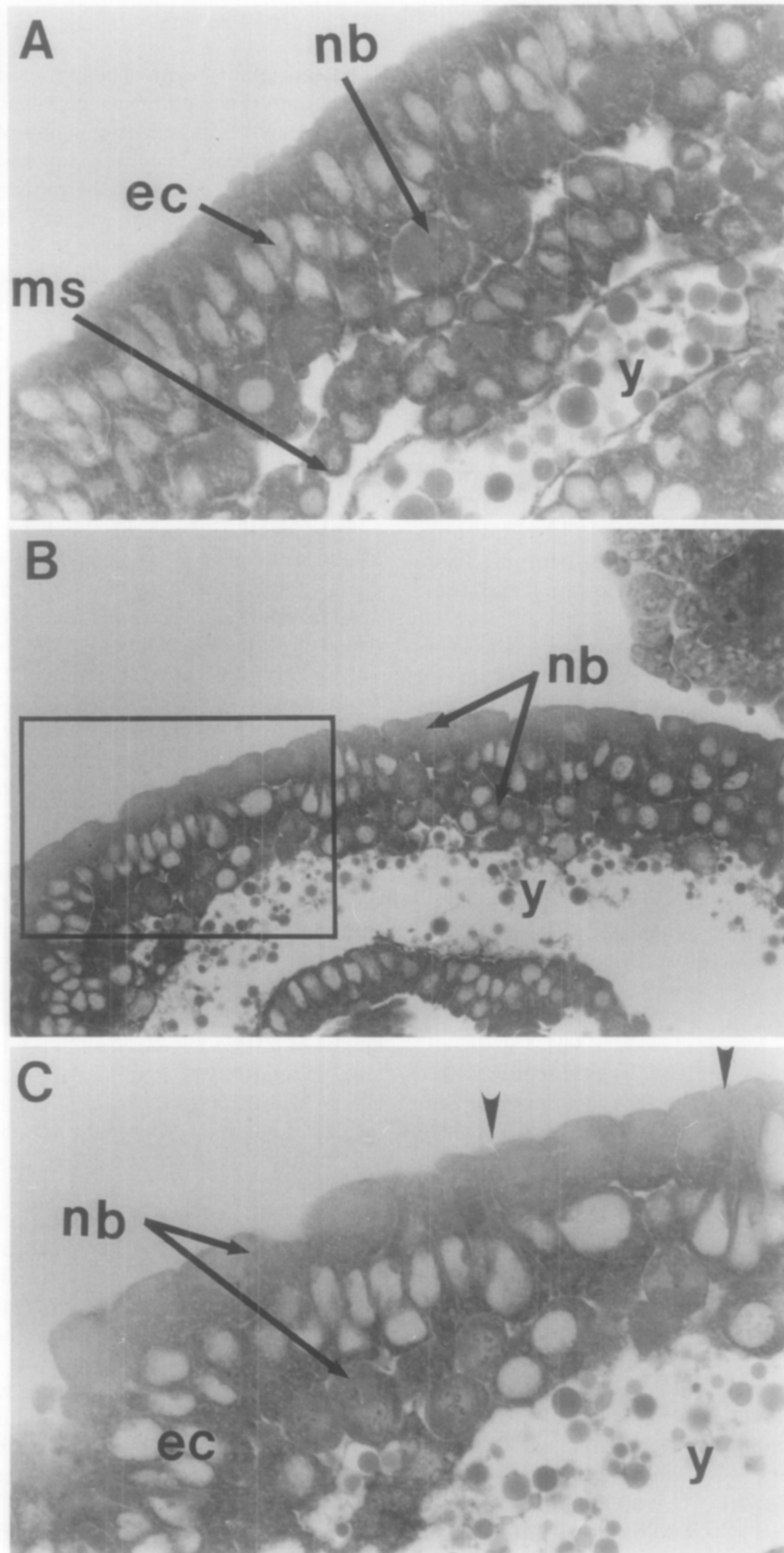

FIG. 5. Neurogenesis defect. (A) A wild-type embryo showing the normal location of the neuroblast layer between the epidermal and mesodermal sheaths at 4 hr of embryonic development. (B) A mutant class 5 embryo of the same age. This embryo is of genotype *l(1)dlg-1*^{M52}/*Y*; it is derived from a homozygous germ line clone for the zygotic lethal allele of *l(1)dlg-1*^{M52}. An extra layer of large cells that resemble neuroblasts is observed at the surface of the ectoderm. (C) An enlargement of the region indicated in (B). Nomenclature: neuroblast, nb; ectodermal, ec; and mesodermal, ms cells; yolk, y.

TABLE 4
ZYGOTIC RESCUABILITY OF THE MATERNAL EFFECT OF AMORPHIC ALLELES OF *l(1)dlg-1*

Mutation	N	N_{unf}	N_{unh} (1)	N_{unh} (2)	N_L	N progeny ♀	N progeny ♂
M52 X +/*Y*	279	87	101	29	62	54	0
M52 X +/*DpY*	162	88	0	20	54	22	28

Note. Females carrying homozygous germ line clones for $l(1)dlg\text{-}1^{M52}$ were crossed to wild-type males or males carrying a copy of $l(1)dlg\text{-}1^{+}$ on both the X and Y chromosomes (male genotype is $+/Dp(1;Y)v^{+}Yy^{+}$). The phenotype of the fertilized eggs was analyzed and classified into two categories: (1) class 5 embryos, and (2) class 1 and 2 embryos. N is the number of eggs analyzed; N_{unf} is the number of unfertilized eggs; N_{unh} is the number of fertilized eggs that have not hatched; N_L is the number of hatched larvae, and N progeny is the number of adults derived from these larvae.

Dissociation of Zygotic Lethality and Female Sterility

In some interallelic combinations of *l(1)dlg-1* alleles (Table 2) trans-heterozygous females are obtained and are fully viable. However, these females exhibit a paternally rescuable maternal effect lethal phenotype that falls within the phenotypic series described for $l(1)dlg\text{-}1^{HF321}$ (Fig. 1). The severity of their phenotype correlates with the expected level of gene activity; e.g., embryos derived from females of genotype $l(1)dlg\text{-}1^{HF321}/l(1)dlg\text{-}1^{v55}$ at 18°C have a class 1 embryonic phenotype, while those of genotype $l(1)dlg\text{-}1^{M35}/l(1)dlg\text{-}1^{1P20}$ exhibit a class 3 phenotype at both 18 and 25°C. Thus, for these interallelic combinations it is possible to separate the zygotic lethality from the maternal effect lethal phenotype.

DISCUSSION

l(1)dlg-1 Is a Complex Locus

The genetics of *l(1)dlg-1* is complex. The nine mutations analyzed at this locus can be divided into four groups based on their complementation pattern (Table 2) and level of gene activity. Only one allele, $l(1)dlg\text{-}1^{XI\text{-}2}$ (group 1), behaves as a true null or amorphic mutation (Muller, 1932); i.e., the complementation pattern is identical to a deficiency of the locus. The complementation pattern of the four group 2 alleles is interesting since they behave as null alleles when tested over a deficiency of the region; however, when tested over $l(1)dlg\text{-}1^{HF321}$ they show negative complementation. Interactions of this kind are typically thought to result from the incorporation of different gene products, encoded by a single locus, into the same multimeric protein such that one subunit disrupts the activity of the other(s). Group 3 alleles give very unusual complementation patterns where full complementation of the zygotic lethality is obtained between a subset of alleles. This most likely reflects the presence of multiple products from the *dlg-1* locus. Finally, two temperature-sensitive mutations, $l(1)dlg\text{-}1^{HF321}$ and $l(1)dlg\text{-}1^{v55}$, have been identified.

$l(1)dlg\text{-}1^{+}$ Is Required throughout Development

Only a few loci have been identified in which mutations result in the formation of large discs. It is assumed that some of these mutations are specifically involved in termination of cell proliferation at the end of disc development (Bryant, 1986). One would expect the genes involved in the termination process to be to be specifically expressed in imaginal cells.

The temperature-shift experiments using $l(1)dlg\text{-}1^{HF321}$ indicate that correct expression of $l(1)dlg\text{-}1^{+}$ is needed throughout imaginal discs' development. Additionally, both the germ line clonal analysis of zygotic lethal alleles (Tables 1 and 4) and the female sterility associated with $l(1)dlg\text{-}1^{HF321}$ homozygous females (Table 3) indicate that $l(1)dlg\text{-}1^{+}$ is expressed maternally. Finally, $l(1)dlg\text{-}1^{+}$ is expressed early during embryonic development since the maternal effect is paternally rescuable to some extent (Tables 3 and 4). These results indicate that $l(1)dlg\text{-}1^{+}$ is expressed maternally as well as zygotically both in embryonic and imaginal cells. It is unclear whether the same gene product is required during these three periods. Interallelic combinations (Table 2) show that it is possible to separate the zygotic lethality from the maternal effect lethal phenotype suggesting that multiple gene products may originate from the *dlg-1* locus. Molecular analysis of the gene will clarify this issue.

Maternal-Zygotic Effect of $l(1)dlg\text{-}1^{+}$

In the absence of both maternal and zygotic $l(1)dlg\text{-}1^{+}$ expression embryos cannot complete embryonic development and have a class 5 embryonic phenotype. The absence of maternal product can be complemented to some extent by the introduction of a wild-type copy of the gene through the sperm. Therefore, $l(1)dlg\text{-}1^{+}$ func-

tion(s) during embryonic development can be provided either maternally or zygotically, indicating that the maternal product is not essential for embryonic development. This rescue is not fully penetrant; however, a large number of morphologically normal adults are recovered. Similarly, the failure of paternal rescue to be fully penetrant has been observed with a number of maternal effect loci, mainly among the neurogenic loci (e.g., *fs(1)pecanex* (Perrimon *et al.*, 1984b) and *fs(1)almondex* (Shannon, 1972)). This very likely represents the variability from embryo to embryo of the amount of wild-type gene product produced zygotically. Using the temperature-sensitive allele *l(1)dlg-1*^{HF321} it has been possible to examine the effect of various dosages of *l(1)dlg-1*$^{+}$ during embryonic development. The defects observed form a phenotypic series clearly showing progressively severe phenotypes and ultimately involving most morphogenetic processes (Figs. 1 and 3 and Table 3).

In the presence of maternal but the absence of zygotic *l(1)dlg-1*$^{+}$ product(s) embryogenesis can be completed. The maternal perdurance allows completion of embryogenesis and permits the observation of lack of *l(1)dlg-1*$^{+}$ gene activity in the mutant larvae. Mutant larvae derived from heterozygous mothers exhibit defects in dividing cells and pupariation. Defects in pupariation have been attributed to no or low levels of ecdysone resulting from a nonfunctional ring gland (Bryant, 1986).

Role of l(1)dlg-1^{+}

The analysis of *dlg-1* indicates that this gene is needed for normal morphogenesis of both the embryo and the adult. Based on the phenotype of mutant embryos, I speculate that the function of the *l(1)dlg-1* locus is to encode product(s) involved in general cell adhesion.

During embryogenesis the absence of the *l(1)dlg-1*$^{+}$ product(s) observed in class 5 embryos (those that are missing both maternal and zygotic *l(1)dlg-1*$^{+}$ gene product(s)) apparently interferes with neurogenesis (Fig. 5). Examination of these embryos by means of plastic sections (Fig. 5) has indicated that there is apparently a larger number of neuroblasts. Cell-cell interactions are known to play a central role during neurogenesis (Doe and Goodman, 1985) and defects in cell adhesion are likely to interfer with normal cell-cell interactions. If more neuroblasts are determined from the neurogenic region and steric hindrance results in segregation in both directions, the defects observed in class 5 embryos are similar (although less extreme) to the defects associated with the neurogenic loci. Neurogenic mutations (Lehman *et al.*, 1983) cause most or all of the cells of the neurogenic region to enter the neural pathway, and result in embryos with a hypertrophied brain and ventral nerve cord. Such loci are thought to be involved in the developmental switch between epidermal and neural differentiation. A major difference between the phenotype of neurogenic mutations and the phenotype of *l(1)dlg-1* embryos is the degeneration of the epidermis in *l(1)dlg-1* embryos. This process does not happen in neurogenic mutations, the residual epidermal cells, though reduced in number, are still capable of secreting cuticle.

Defects in cell adhesion can also account for the abnormalities in mesodermal and epidermal tissues observed during embryogenesis of *l(1)dlg-1*^{HF321} mutants (Fig. 4). It is likely that abnormal adhesivity would perturb the attachment of muscles to apodemes as well as dorsal closure.

Mutations at another locus, *lethal(2)giant larvae* (*l(2)gl;* Hadorn, 1961; Gateff and Schneiderman, 1967; Hanratti, 1984a,b) which result in tumorous imaginal discs overgrowth support the speculation that *l(1)dlg-1*$^{+}$ may be involved in cell adhesion. *l(2)gl* has been cloned (Mechler *et al.*, 1985) and the deduced amino acid sequence predicts a 130-kDa protein that has sequence similarities with cell adhesion molecules (Lutzelschwab *et al.*, 1987). Studies with antibodies raised against the *l(2)gl* gene product indicate that the protein is associated with membranes and is likely to be involved in cell-cell interactions (Schwab *et al.*, 1987). The maternal effect of *l(2)gl* is not known. However, the gene is heavily transcribed during oogenesis and embryogenesis (Mechler *et al.*, 1985) and thus it most likely plays a role during embryonic development. Based on the analysis of the maternal effect of *l(1)dlg-1* it is tempting to predict that *l(2)gl* will exhibit a maternal effect lethal phenotype, possibly similar to the one observed for *l(1)dlg-1*.

I am grateful to Drs. G. Lefevre, B. Geer, R. Voelker, and P. Bryant and the Pasadena stock center for sending us stocks; L. Perkins, R. Binari, D. Smouse for comments on the manuscript; D. Woods and P. Bryant for sharing unpublished informations; B. Noll and K. Maier for excellent technical assistance; and A. P. Mahowald in whose laboratory these experiments were started. This work was supported by the Howard Hughes Medical Institute.

REFERENCES

BRYANT, P. J. (1986). Experimental and genetic analysis of growth and cell proliferation in *Drosophila* imaginal discs. *In* "Symposia for Developmental Biology" (J. Gall, Ed.), A. R. Liss, New York.

BUSSON, B., GANS, M., KOMITOPOULOU, K., and MASSON, M. (1983). Genetic analysis of three dominant female sterile mutations located on the *X*-chromosome of *Drosophila melanogaster. Genetics* **105**, 309-325.

CAMPOS-ORTEGA, J. A., and HARTENSTEIN, V. (1985). "The Embryonic Development of *Drosophila melanogaster.* Springer-Verlag, Berlin/Heidelberg/New York.

CRAYMER, L., and ROY, E. (1980). New mutants. *Drosophila Inform. Serv.* **55**, 200-204.

DEQUIN, R., SAUMWEBER, H., and SEDAT, J. W. (1984). Proteins shifting from the cytoplasm into the nucleus during early development of *Drosophila melanogaster. Dev. Biol.* **104,** 37-48.

DOE, C. Q., and GOODMAN, C. S. (1985). Early events in insect neurogenesis: II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Dev. Biol.* **111,** 193-205.

GATEFF, E. (1978). Malignant and benign neoplasms of *Drosophila melanogaster. In* "The Genetics and Biology of Drosophila" (M. Ashburner and T. R. F. Wright, Eds.), Vol 2B, pp. 152-276. Academic Press, New York.

GATEFF, E., and SCHNEIDERMAN, H. A. (1967). Developmental studies of a new mutation of *Drosophila melanogaster: lethal malignant brain tumor l(2)gl^4. Amer. Zool.* **7,** 760.

GEER, B. W., LISHWE, T. D., and MURPHY, K. G. (1983). Male fertility in *Drosophila melanogaster:* Genetics of the *vermilion* region. *J. Exp. Zool.* **225,** 107-118.

HADORN, E. (1961). "Developmental Genetics and Lethal Factors." Methuen, New York.

HANRATTI, W. P. (1984a). Isolation and characterization of temperature sensitive *lethal(2) giant larvae* alleles. I. Mutagenesis and larval pathology. *Wilhelm Roux's Arch. Dev. Biol.* **193,** 90-97.

HANRATTI, W. P. (1984b). Isolation and characterization of temperature sensitive *lethal(2) giant larvae* alleles. II. Temperature-sensitive expression of the imaginal disc neoplasm. *Wilhelm Roux's Arch. Dev. Biol.* **193,** 98-107.

LEHMANN, R., JIMENEZ, F., DIETRICH, U., and CAMPOS-ORTEGA, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol.* **192,** 62-72.

LINDSLEY, D. L. and GRELL, E. H. (1968). *Genetic variations of Drosophila melanogaster. Carnegie Inst. Washington Publ.* No. 627.

LUTZELSCHWAB, R., KLAMBT, C., ROSSA, R., and SCHMIDT, O. (1987). A protein product of the *Drosophila* recessive tumor gene, *l(2)giant gl,* potentially has cell adhesion properties. *EMBO J.* **6,** 1791-1797.

MAHOWALD, A. P., CAULTON, J. H., and GEHRING, W. J. (1979). Ultrastructural studies of the oocytes and embryos derived from females carrying the *grandchildless* mutation in *Drosophila subobscura. Dev. Biol.* **69,** 451-465.

MECHLER, B. M., MCGINNIS, W., and GEHRING, W. J. (1985). Molecular cloning of *lethal(2) giant larvae,* a recessive oncogene of *Drosophila melanogaster. EMBO J.* **4,** 1551-1557.

MITCHISON, T. J., and SEDAT, J. W. (1983). Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99,** 261-264.

MULLER, J. H. (1932). Further studies on the nature and cause of gene mutations. *Proc. 6th Int. Congr. Genet.* **1,** 213-272.

PERRIMON, N. (1984). Clonal analysis of dominant female sterile, germ line dependent mutations in *Drosophila melanogaster. Genetics* **108,** 927-939.

PERRIMON, N., ENGSTROM, L., and MAHOWALD, A. P. (1984a). The effects of zygotic lethal mutations on female germ-line functions in *Drosophila. Dev. Biol.* **105,** 404-414.

PERRIMON, N., ENGSTROM, L., and MAHOWALD, A. P. (1984b). Developmental genetics of the 2E-F region of the *Drosophila X*-chromosome: A region rich in "developmentally important" genes. *Genetics* **108,** 559-572.

PERRIMON, N., and GANS, M. (1983). Clonal analysis of the tissue specificity of recessive female-sterile mutations in *Drosophila melanogaster* using a dominant female-sterile mutation *fs(1)K1237. Dev. Biol.* **100,** 365-373.

PERRIMON, N., and MAHOWALD, A. P. (1986). *l(1)hopscotch,* a larval-pupal zygotic lethal with a specific maternal effect phenotype on segmentation in *Drosophila. Dev. Biol.* **118,** 28-41.

SHANNON, M. P. (1972). Characterization of the female-sterile mutant *almondex* of *Drosophila melanogaster. Genetics* **43,** 244-256.

SHEARN, A. (1978). Mutational dissection of imaginal disc development. *In* "The Genetics and Biology of Drosophila" (M. Ashburner and T. R. F. Wright, Eds.), Vol 2C, pp. 443-510. Academic Press, New York.

STEWART, M., MURPHY, C., and FRISTROM, L. (1972). The recovery and preliminary characterization of X-chromosome mutants affecting imaginal discs of *Drosophila melanogaster. Dev. Biol.* **27,** 71-83.

TURNER, F. R., and MAHOWALD, A. P. (1976). Scanning electron microscopy of *Drosophila* embryogenesis. I. Structure of the egg envelopes and the formation of the cellular blastoderm. *Dev. Biol.* **50,** 95-108.

TURNER, F. R., and MAHOWALD, A. P. (1977). Scanning electron microscopy of *Drosophila* embryogenesis. II. Gastrulation and segmentation. *Dev. Biol.* **57,** 403-416.

VAN DER MEER, J. (1977). Optical clean and permanent whole mount preparations for phase contrast microscopy of cuticular structures of insect larvae. *Drosophila Inform Serv.* **52,** 160.