DEVELOPMENTAL BIOLOGY 119, 587–600 (1987)

Multiple Functions of Segment Polarity Genes in *Drosophila*

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Received July 14, 1986; accepted in revised form September 26, 1986

l(1)dishevelled (*l(1)dsh*) is a late zygotic lethal mutation that exhibits a rescuable maternal effect lethal phenotype. *l(1)dsh/Y* embryos, derived from females possessing a homozygous *l(1)dsh* germline clone, exhibit a segment polarity embryonic phenotype. Analysis of the development of these embryos indicates: (1) that segmental boundaries do not form although the correct number of tracheal pits is formed; (2) that pockets of cell death occur between the tracheal pits; and (3) that *engrailed* expression becomes abnormal during germ band shortening. We propose that, in the absence of both maternal and zygotic expression of *l(1)dsh*$^+$, cells from each posterior compartment die. Subsequently, cells from the anterior compartment must rearrange their positional values to generate the segment polarity phenotype. We have compared the phenotype of *l(1)dsh* with the phenotype of five other segment polarity loci: four embryonic lethals [*l(1)armadillo*, *l(2)gooseberry*, *l(2)wingless*, and *l(3)hedgehog*]; and the late zygotic lethal, *l(1)fused*. Only *l(2)wingless* embryos exhibit early segmentation defects similar to those found in *l(1)dsh/Y* embryos derived from homozygous germline clones. In contrast, segmentation is essentially normal in *l(1)armadillo*, *l(2)gooseberry*, *l(3)hedgehog*, and *l(1)fused* embryos. The respective maternal and zygotic contribution and the roles of the segment polarity loci for the patterning of the embryo and the adult are discussed.

INTRODUCTION

Both *Drosophila* embryos and adults are composed of a set of repetitive units each of which is the composite of two different lineages (polyclones), one for the anterior structures of the segment (the anterior compartment) and one for the posterior structures (the posterior compartment), (Garcia-Bellido *et al.*, 1973). Repetitive units are morphologically evident in the *Drosophila* embryo shortly after tracheal pit formation (Turner and Mahowald, 1977). Recently, Ingham *et al.* (1985a) have shown that the first repetitive units that can be detected in the embryo are not segmental but parasegmental (Martinez-Arias and Lawrence, 1985). Each parasegment is the composite of an anterior and a posterior compartment belonging to different segmental units. Soon after their formation, the parasegmental units disappear and segmental boundaries are formed, coinciding with the position of the tracheal pits (Keilin, 1944; Ingham *et al.*, 1985a; Petschek *et al.*, 1986).

Systematic searches for genes involved early in establishing the segmental pattern have led to the identification of three classes of embryonic lethal loci: the gap, pair rule and segment polarity genes (Nusslein-Volhard and Wieschaus, 1980). Mosaic analyses (Gergen and Wieschaus, 1985, 1986) of some of these genes suggest that their activity is required in specific regions of the developing embryo. Additional evidence for localized gene activity is provided by the detection of localized transcripts of a series of loci: *fushi tarazu* (Hafen *et al.*, 1984; Carrol and Scott, 1985); *engrailed* (Kornberg *et al.*, 1985; DiNardo *et al.*, 1985); *Kruppel* (Knipple *et al.*, 1985); and *hairy* (Ingham *et al.*, 1985b).

The segment polarity phenotype displays a repeated pattern defect in which part of each segment is deleted and a mirror-image duplication of the remaining pattern element forms (Nusslein-Volhard and Wieschaus, 1980). Mutations at five loci produce an embryonic segment polarity phenotype *l(1)armadillo* (Wieschaus *et al.*, 1984), *l(2)wingless*, *l(2)gooseberry*, *l(3)hedgehog*, *l(4)cubitus interruptus* (Nusslein-Volhard and Wieschaus, 1980). Lack of function mutations at a sixth locus, *l(1)fused*, die during late pupal stages. Flies, however, carrying mutations at the *fused* locus with residual gene activity (hypomorphs) are homozygous viable but exhibit a partially rescuable maternal effect embryonic lethality (Counce, 1956; Perrimon *et al.*, 1986; this study). Lack of both maternal and zygotic activity of *fused* leads to embryos with a segment polarity phenotype (Nusslein-Volhard and Wieschaus, 1980). In this paper, we describe *l(1)dishevelled*, which is a larval lethal locus that produces a segment polarity phenotype in the absence of both maternal and zygotic activity. We have examined the morphological events that lead to, or are associated with, the segment polarity phenotype and we discuss the roles of these genes in the patterning of the embryo and adult.

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0012-1606/87 $3.00

MATERIALS AND METHODS

Strains. The origin of the mutations used in this study are listed in Table 1. Stocks of *l(2)gsb*^{IIX62}, *l(2)wg*^{IG22}, and *l(3)hh*^{6L93} were obtained from the Bowling Green Stock Center. The deficiency of *fused, Df(1)N19* was obtained from the Pasadena Stock Center. *l(1)arm*^{XM19} and *l(1)arm*^{XK22} were obtained from E. Wieschaus. *dsh*^{1}, *l(1)dsh*^{M20}, and *l(1)dsh*^{v26} were obtained from D. Sponaugle and *l(1)dsh*^{VA153} is from G. Lefevre.

The *X*-linked dominant female sterile mutation; *Fs(1)K1237* (Busson *et al.*, 1983; Perrimon, 1984), is maintained in an attached-*X* stock: *C(1)DX, y f/Y* females crossed to *Fs(1)K1237 v*^{24}*/Y* males.

Descriptions of stocks and balancers, unless identified in the text, can be found in Lindsley and Grell (1968).

All experiments were conducted at 25°C on standard *Drosophila* medium.

Germline clones analysis. As previously described, germline clones of *X*-linked zygotic lethals were induced according to the dominant female sterile technique utilizing the mutation *Fs(1)K1237* (or *Ovo*^{D1}) (Perrimon, 1984; Perrimon *et al.*, 1984). Flies were irradiated with a constant dose of 1000 rad (γ-ray machine; Model GR-9 Co-60 irradiator) at the end of the first larval instar stage. These conditions generate from 4 to 7% mosaic females. In all experiments, flies carrying germline clones were analyzed individually to allow the identification of distal mitotic recombination events (Perrimon and Gans, 1983).

Analysis of zygotic phenotypes. Embryos were examined by one of five methods: (1) whole embryonic cuticles were prepared according to the Hoyer's technique of van der Meer (1977); (2) histological sections were prepared as described by Mahowald *et al.* (1979); (3) scanning electron micrographs (SEM) were prepared as described by Turner and Mahowald (1976); (4) the nervous system was visualized by staining for acethylcholinesterase (Brown and Schubiger, 1981); and (5) embryos were stained with an antibody against *engrailed* protein as described by DiNardo *et al.* (1985). The vitelline membranes were removed according to the technique of Mitchison and Sedat (1983) modified by Dequin *et al.* (1984).

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

TABLE 1

LOCATION, ORIGINS, LETHAL PHASE, AND MATERNAL EXPRESSION OF THE SEGMENT POLARITY MUTATIONS

					Maternal expression		
	Location	Origin	Ref.	LP	*N*	N_{GLC}	Phenotype
dsh^{1}	10B6-7	MMS	(1)	—	500	46	NME
dsh^{v26}		EMS	(2)	L	400	35	MELR
dsh^{M20}		ENU	(3)	L	600	41	MELR
dsh^{VA153}		EMS	(4)	L	200	10	MELR
dsh^{9PP3}		EMS	(5)	L	350	21	MELR
dsh^{9PP6}		EMS	(5)	L	300	14	MELR
fu^{2P}	17D-E	EMS	(5)	—	400	27	MELR, AO
fu^{9P2}		EMS	(5)	P-A	500	42	MELR, AO
fu^{1PP7}		EMS	(5)	P-A	550	47	MELR, AO
arm^{XM19}	2B15-17	EMS	(6)	E	300	19	AO
arm^{XK22}		EMS	(6)	E	250	15	AO
wg^{IG22}	2. 30*	EMS	(7)	E	NT		
gsb^{IIX62}	60E9-F1	EMS	(7)	E	NT		
hh^{6L93}	94E	EMS	(8)	E	NT		

Note. Origin: methyl-methanesulfonate (MMS) ethyl-methanesulfonate (EMS), *N*-ethyl-*N*-nitrosourea (ENU). *References:* (1) Lindsley and Grell, 1968; (2) Greer *et al.*, 1983; (3) Voelker, unpublished observation; (4) Lefevre, unpublished observation. (5) this study; (6) Wieschaus *et al.*, 1984; (7) Nusslein-Volhard *et al.*, 1984; (8) Jurgens *et al.*, 1984. In screens for late (larval-pupal period) *X*-linked lethal mutations using ethyl-methanesulfonate as the mutagen, we induced two *dishevelled* alleles (*l(1)dsh*^{9PP3} and *l(1)dsh*^{9PP6}) as well as three *fused* alleles (*fu*^{2P}, *fu*^{9P2} and *fu*^{1PP7}), (Perrimon, unpublished). *l(1)fu*^{9P2} and *l(1)fu*^{1PP7} are late pupal-early adult lethals; *fs(1)fu*^{2P} was selected because of its visible phenotype. The lethal phase (LP) of each mutation is indicated (E, embryonic; L, larval; P, pupal, and A, adult, which die at emergence). Maternal expression was analyzed by germline clone analysis. *N* is the number of females analyzed and N_{GLC} corresponds to the number of females possessing a germline clone. The results are presented as: NME, no maternal effect; MELR, rescuable maternal effect; and, AO, abnormal oogenesis.

* Refers to the meiotic location.

RESULTS

Genetics of l(1)dishevelled

The first allele of *dishevelled* was induced with methyl-methanesulfonate (Lindsley and Grell, 1968) by Fahmy in 1956, and will be referred to as *dsh*^{1}. Subsequently, recessive, lethal alleles were isolated (*l(1)dsh*^{M20}, *l(1)dsh*^{v26}, *l(1)dsh*^{VA153}, *l(1)dsh*^{9PP3}, and *l(1)dsh*^{9PP6}, see Table 1 for references). The recessive lethals will be referred to as *l(1)dsh*. *l(1)dsh* maps on the *X*-chromosome at the meiotic position 34.5. Cytologically the locus maps in bands 10B5-7 of the salivary gland chromosomes and is located between *l(1)VE874* and *l(1)hopscotch* (Perrimon and Mahowald, 1986b).

The viable allele, *dsh*^{1} shows reduced viability under both hemizygous male and homozygous female conditions (Table 2). However, the expected number of hemizygous *dsh*^{1}*/Df* female progeny are obtained. We have recombined the original *dsh*^{1} chromosome with both *raspberry*(*ras*, 1-32.8) and *miniature* (*m*, 1-36.1), but the recombined *dsh*^{1} chromosome still shows poor viability in either hemi or homogzygous conditions. Since *dsh*^{1}*/Df* is fully viable, we assume that a second site mutation,

TABLE 2
LETHAL PHASES

	Lethality (%)			Mutant adults (%)	
	E	L	P	♂	♀
$+/dsh^1 \times +/Y$	2	1	11	13	
$+/dsh^1 \times dsh^1/Y$	1	0	22	14	11
$+/dsh^1 \times Df/DpY$	1.5	0	4		22
$dsh^1/dsh^1 \times +/Y$	2.5	1	2	44.5	
$dsh^1/dsh^1 \times Df/DpY$	4	1.5	1		50.5
$+/dsh^{M20} \times +/Y$	3	20	3	0	
$+/dsh^{M20} \times dsh^{M20}/DpY$	2	19	4		0
$+/dsh^{M20} \times Df/DpY$	4	21	2		0
$+/dsh^1 \times dsh^{M20}/DpY$	.5	0	1		25
$+/dsh^1 \times dsh^{v26}/DpY$	1.5	3	6		16
$+/dsh^1 \times dsh^{VA153}/DpY$	2.5	.5	1.5		24
$+/dsh^1 \times dsh^{9PP3}/DpY$	1	1	2		23
$+/dsh^1 \times dsh^{9PP6}/DpY$	3	.5	4		24
$+/dsh^{M20} \times dsh^{v26}/DpY$	3.5	20	4		0
$+/fu^{2P} \times +/Y$	2	0	0	24	
$+/fu^{9P2} \times +/Y$	1	0	4	22*	
$+/fu^{1PP7} \times +/Y$	0	0	16	9*	
$+/fu^{2P} \times fu^{2P}/Y$	1	0	21	25	4*
$+/Df(1)N19 \times fu^{2P}/Y$	22	3	24		1.5*

Note. In each cross (indicated in the left-most colum) more than 300 fertilized eggs were analyzed. The percentage of offspring dying during embryonic (E), larval (L), and pupal (P) stages is indicated. Similarly the percentage of male and female mutant adults recovered is shown. The percentage of progeny dying at the embryonic stage was calculated as N_E/N, where N_E represents the number of dead embryos and N the total number of fertilized eggs. Similar calculations were done for the other stages. *FM7* was used in females as the "+" chromosome, and *y f* in males. *Df* referred to *Df(1)N71 (Df(1)*10B5-D4) and *DpY* to v^+Yy^+ (*Dp(1;Y)* 9F3 to 10C1) (Craymer and Roy, 1980). Adult $l(1)fu^{9P2}$ and $l(1)fu^{1PP7}$ males, derived from heterozygous mothers, exhibit an extreme adult *fused* phenotype with veins L3 and L4 fused along their entire length and only two scutellar bristles present. These males die shortly after emergence. We conclude that amorphs at the *fused* locus are zygotic lethals and that when *fused* is mutated to a hypomorphic condition it behaves as a female sterile mutation (see Perrimon *et al.*, 1986 for other examples of hypomorphic lethal loci showing female sterility). Females homozygous for $fs(1)fu^{2P}$ have a lower viability than $fs(1)fu^{2P}/Y$ males. Extensive recombination analyses indicates that the two lethal alleles are not associated with second site mutation(s) affecting viability. The lethal phase of the trans-heterozygote $fs(1)fu^{2P}$ over a deficiency of the locus (*Df(1)N19*) indicates that *fused* is indeed a zygotic lethal and that $fs(1)fu^{2P}$ is hypomorphic; i.e., an earlier lethal phase is observed in flies carrying $fs(1)fu^{2P}$ over *Df(1)N19*.

* Refers to adults with poor viability which usually die at emergence.

located between *ras* and *m*, is responsible for the poor viability. The remaining features of the previously described phenotype of dsh^1 ("thoracic hairs deranged, wings divergent and blistered, eyes ellipsoid, male fertile, and female sterile") (Lindsley and Grell, 1968) have been verified by us, except that female sterility (Fahmy, 1956) is no longer associated with the mutation. It is possible that the original chromosome studied by Fahmy also had a second site female sterile mutation.

The lethal phase of either hemizygous male or homozygous female larvae, produced from heterozygous mothers, for any of the five zygotic lethal mutations occurs primarily during second to early third instar larval stages; we only show the results with one allele, $l(1)dsh^{M20}$ in Table 2, but the data are similar for the other four alleles ($l(1)dsh^{v26}$, $l(1)dsh^{VA153}$, $l(1)dsh^{9PP3}$, and $l(1)dsh^{9PP6}$). These larvae can be found alive several days after their wild-type siblings have pupated. Each of these alleles show the same lethal phase when tested as hemizygotes with a deficiency (Table 2), a result suggesting they act as null alleles for the gene (Muller, 1932). Analysis of each of these lethal alleles in transallelic combinations with other lethal alleles failed to uncover any complementation among the mutations. In contrast to these lethal alleles, dsh^1 fully complemented the larval lethal phase of the null alleles, i.e., females heterozygous for dsh^1 and any of the lethal alleles, displayed the adult *dsh* phenotype and were fertile (Table 2).

The Maternal Effect of l(1)dsh

To address the role of *l(1)dsh* during oogenesis we utilized the dominant female sterile technique (Perrimon *et al.*, 1984a) to prepare germline clones of the five larval lethal alleles (all of them give similar results so that we only show the results with one allele $l(1)dsh^{v26}$, Table 3). Females, possessing homozygous germline clones when crossed to wild-type males, produce two classes of phenotypically different embryos. One half of the embryos

TABLE 3
GERMLINE CLONE ANALYSIS

			Lethality (%)		Progeny (%)	
	N	N_{unf}	E	L-P	♂	♀
$dsh^{v26} \times +/Y$	384	52	52	1	0	47
$dsh^{v26} \times +/DpY$	210	28	3	4	46	47
$dsh^{v26} \times Df/DpY$	237	18	48	2	50	0
$dsh^{v26} \times dsh^1/Y$	257	10	49	4	0	47
$fu^{9P2} \times +/Y$	760	152	68.5	4	0	27.5
$fu^{1PP7} \times +/Y$	1205	163	66	31	0	3

Note. Females possessing a germline clone for various alleles were crossed to males of various genotypes. The number of eggs analyzed in each cross (N), and the number of unfertilized (N_{unf}) is shown. the percentage of lethality (E, embryonic; and L-P, larval or pupal) and of all emerged male or female adults are shown. The percentage of progeny dying at the embryonic stage was calculated as $N_E - N_{unf}/N - N_{unf}$, where N_E represents the number of unhatched embryos. Similar calculations were done for the other stages.

(*l(1)dsh/+*) are normal and differentiate into morphologically normal and fertile females. Male embryos (*l(1)dsh/Y*) exhibit a unique and fully penetrant phenotype. In late embryos only ventral cuticle is present, covered with a lawn of setae (Fig. 1F). These embryos lack dorsal cuticle, posterior spiracles, and filzkorper material. Throughout the text we will refer to these embryos as *dishevelled* embryos. The ventral cuticle resem-

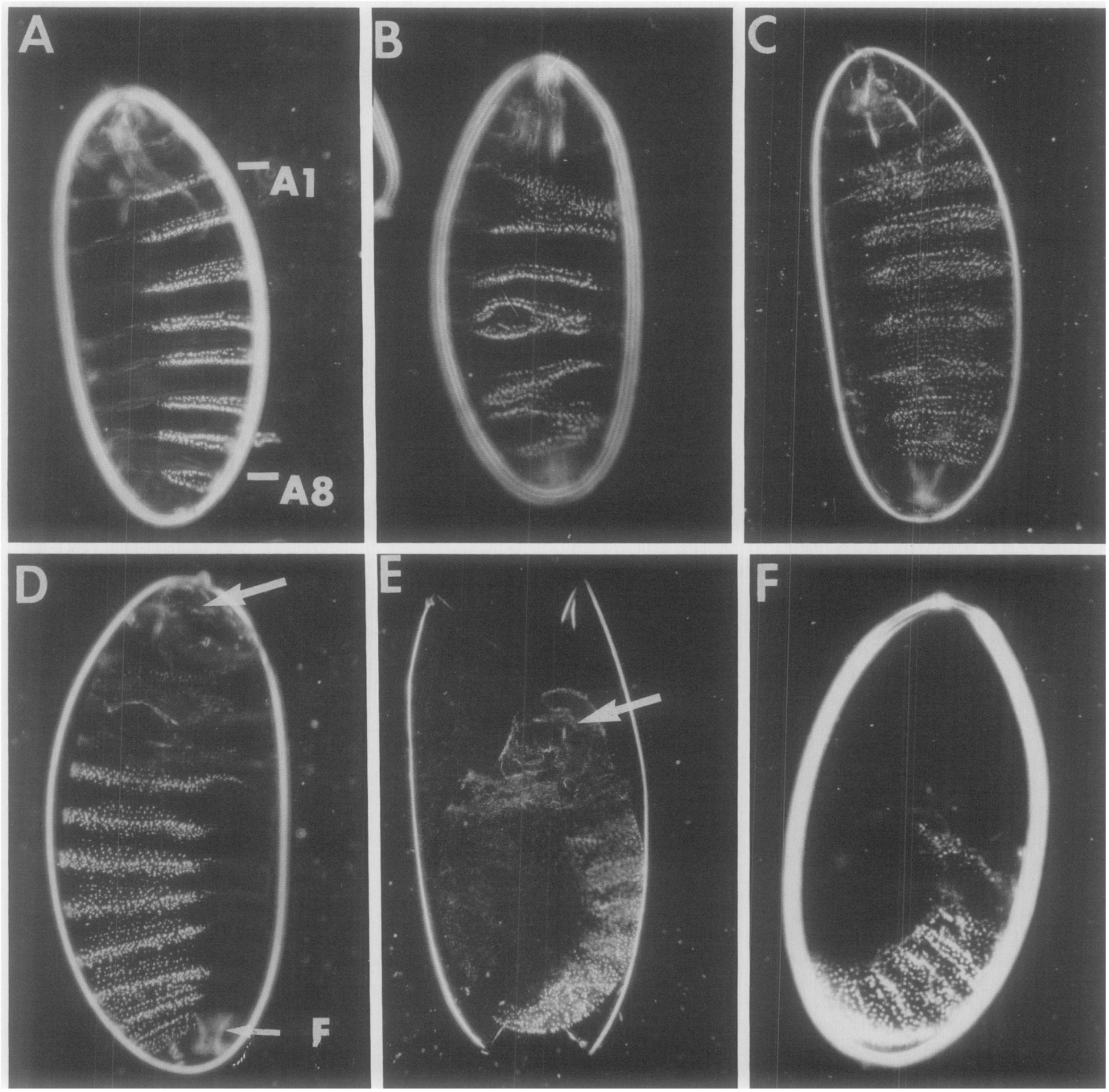


FIG. 1. Cuticle phenotypes of reverse polarity loci. (A) Is a dark field micrograph of a wild-type embryo. Note the eight abdominal denticle belts (A1 through A8). (B)Is a rescued *fused* embryo *l(1)fu/+* derived from a homozygous *l(1)fu* germline clone. (C) Is a nonrescued *fused* embryo. The head is involuted in this embryo and each segment exhibits the segment polarity phenotype. Some embryos homozygous for *l(2)gooseberry* or *l(1)armadillo* also exhibit the phenotype depicted in (C). (D) Defects in the head (arrow) are observed in some embryos homozygous for *l(2)gooseberry*. Some *l(1)armadillo* embryos are similar. (E) Embryos homozygous for *l(2)wingless* exhibit an extreme phenotype with complete lack of head structures (arrow) and filzkorper. (F) *l(1)dsh/Y* embryos derived from germline clones of *l(1)dsh* exhibit a very strong segment polarity phenotype with no detectable dorsal cuticle and head structures. Anterior is up in all figures.

bles that observed with mutations of segment polarity loci (Nusslein-Volhard and Wieschaus, 1980) which are characterized by the absence of naked cuticle in thoracic and abdominal segments (see below and Fig. 1). There is no effect of temperature on this maternal effect.

To demonstrate the full rescuability of the maternal effect of *l(1)dsh*, two further crosses were carried out (Table 3). Females carrying germline clones for any of the five larval lethals were crossed either to males carrying *l(1)dsh*$^+$ on both the *X* and *Y* chromosome (*+/DpY* males) or to males carrying a deficiency of *l(1)dsh* on the *X*-chromosome and a duplication of the region on the *Y*-chromosome (*Df/DpY* males). The first set of crosses indicates that both male and female embryos respond similarly to the paternal rescue. The second set of crosses shows that *l(1)dsh/Df* hemizygous embryos derived from a germline clone exhibit the same phenotype as *l(1)dsh/Y* embryos. If *l(1)dsh/Df* embryos had displayed a more severe embryonic phenotype than *l(1)dsh/Y* or *l(1)dsh/l(1)dsh*, then the lethal allele tested should have some residual activity. Because no differences were obtained we conclude that the five larval lethal alleles are lack of function mutations (amorphs) by this criterion. This agrees with our previous results on the zygotic lethal phase.

As already mentioned, *dsh*^{1}/*dsh*^{1} and *dsh*^{1}/*Df* females are fertile indicating that *dsh*^{1} does not exhibit a maternal effect. However, to test whether *dsh*^{1} had a reduced ability to rescue the maternal deficiency of *l(1)dsh*, we prepared germline clones of larval lethal alleles and crossed these females to *dsh*^{1}/*Y* males (Table 3). Since the expected number of *dsh*^{1}/*l(1)dsh* adult female progeny were obtained (Table 3), *dsh*^{1} must behave like the wild-type allele with regard to the early zygotic rescue. These results suggest that two functions exist at the *dishevelled* locus: (1) an early function responsible for the maternal effect and the larval lethal phenotype; and (2) a late function responsible for the adult phenotype. The *dsh*^{1} mutation appears amorphic for the late function and wild type for the early one. In contrast the five zygotic lethal alleles behave as amorphic for both early and late functions. Alternatively, it is possible that *dsh*^{1} is a weak allele that has sufficient activity for normal embryonic development but not for normal development to adulthood.

Embryonic Defects Associated with the Maternal Effect of l(1)dsh

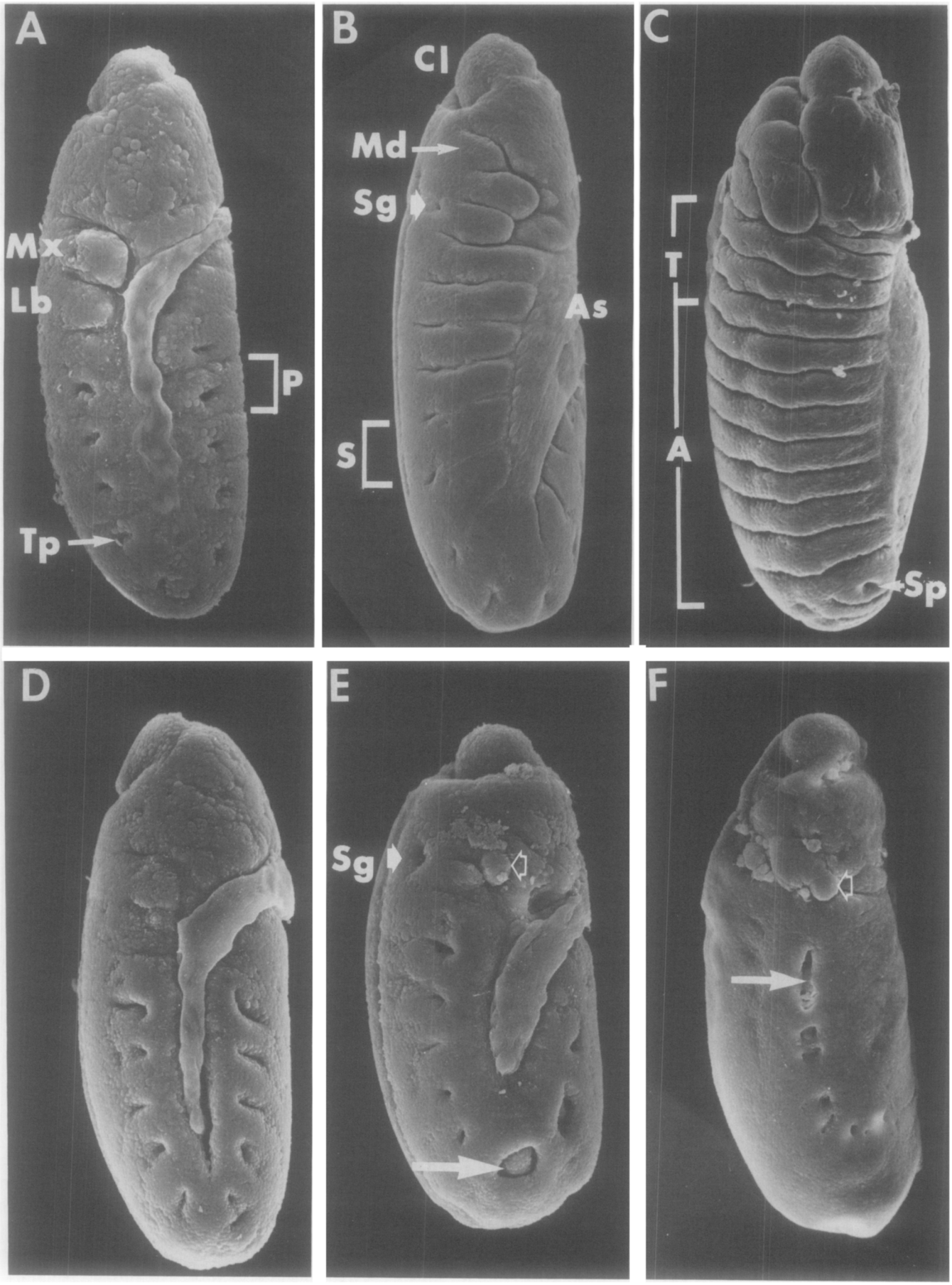
The embryonic phenotype associated with the maternal effect of *l(1)dsh* was analyzed histologically and by scanning electron microscopy. In *l(1)dsh/Y* embryos derived from homozygous germline clones both gastrulation and germ band elongation appears normal (results not shown). The first externally visible defects occur at the time of tracheal pit formation (6–7 hr). The correct number of tracheal pits form but the maxillary and labial head segments appear to be missing and parasegmental boundaries do not form (compare Figs. 2D with 2A). At a later stage, structures resembling the maxillary appendage are occasionally present (Fig. 2E). The salivary gland invaginations occur normally although they are wider and deeper than in wild-type embryos. Cell death organized in pockets located near the tracheal pits (Figs. 6A, C) can already be seen histologically at 6 hr. Although the pockets of cell death are in the mesodermal layers (Figs. 6A, C), occasionally cell death is seen to extend into the hypoderm (Fig. 6B). Extensive ectodermal cell death would not be detected histologically if these dying cells were rapidly moved out of the ectoderm layer.

The next morphological defect observed is the lateral fusion of the tracheal pits (Fig. 2E) and the absence of segmental boundaries which should arise near the tracheal pits (Keilin, 1944; Ingham *et al.*, 1985a; Dinardo *et al.*, 1985). Subsequently, *dishevelled* embryos become increasingly more abnormal. Deep lateral furrows form corresponding to the fusion of the patent tracheal pits (Fig. 2F). At 9 hr, cell death in the mesoderm becomes more prominent and by 13 hr most of the segmental and visceral mesoderm is necrotic. The endodermal lining of the gut fails to differentiate. Dorsal closure and head involution do not occur. Amnioserosa cells start to degenerate at 9 hr and are necrotic by 14 hr (Figs. 3B and C). These defects lead to the protrusion of gut structures dorsally and of the brain anteriorly (Figs. 4C, D, 5B). At the time when the cuticle differentiates, setae are produced in a reverse polarity orientation on the entire ventral surface of the embryo (Fig. 4C). This setal orientation can be seen more clearly in Fig. 4G.

Interestingly, the nervous system does not appear to be affected in early *l(1)dsh/Y* embryos derived from homozygous germline clones. At 8–9 hr the ventral cord (visualized by staining for acetylcholine-esterase) appears correctly organized in neuromeres (result not shown) and no obvious cell death is detectable. By 12 hr the ventral cord appears disarranged possibly as a result of the extensive defects in other tissues.

Expression of engrailed in Embryos Derived from l(1)dsh Homozygous Germline Clones

Both genetic (Kornberg, 1981) and molecular evidence (Kornberg *et al.*, 1985; DiNardo *et al.*, 1985) indicates that the function of the *engrailed* gene product is restricted to cells located within the posterior compartment of every segment. Since *l(1)dsh/Y* embryos derived from homozygous germline clones are missing the naked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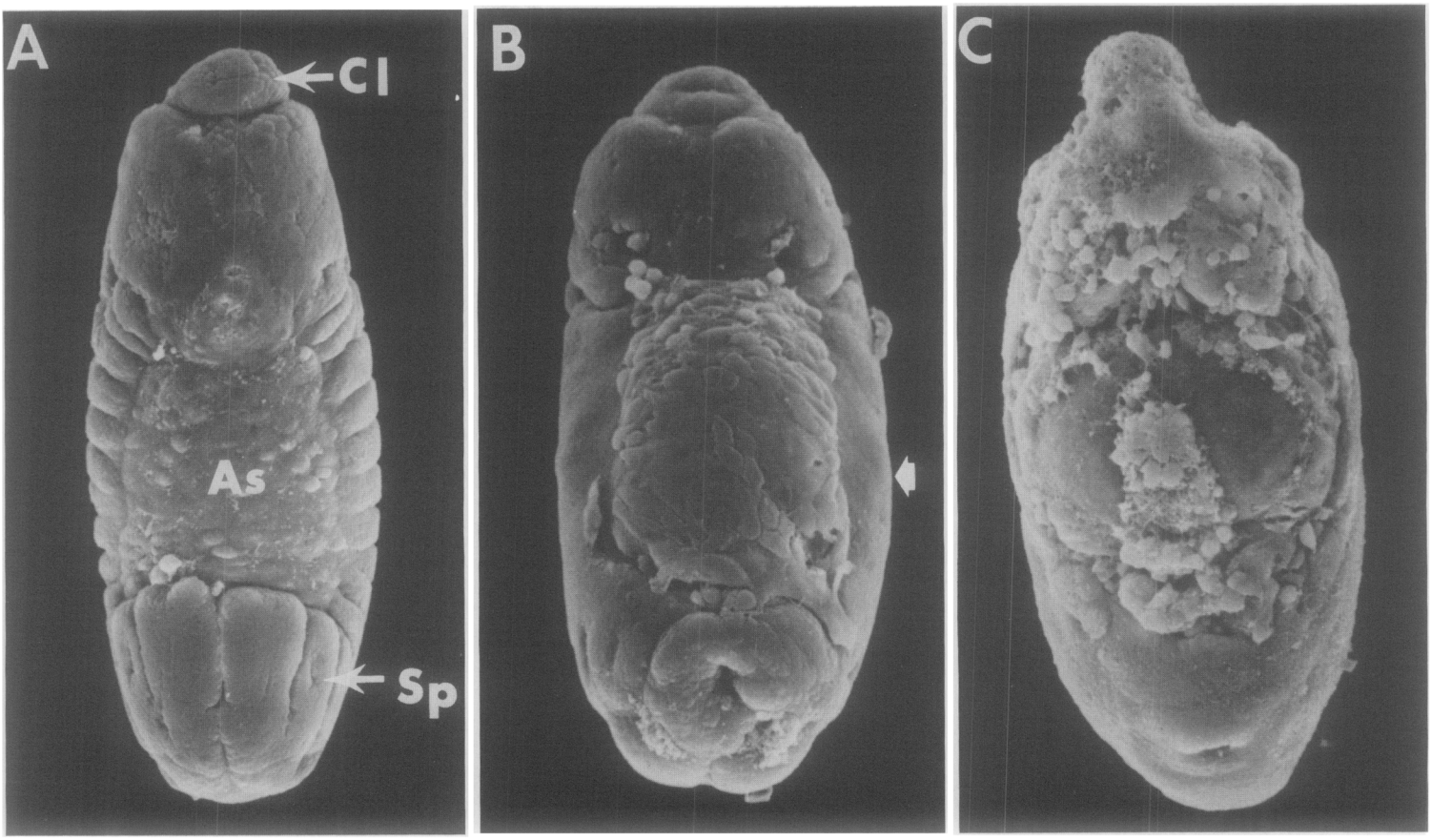

FIG. 3. Dorsal closure defects in embryos derived from germline clones of *l(1)dishevelled*. (A) Is a SEM micrograph of the dorsal aspect of a wild-type embryo at 9 hr of development. In (B) and (C) are two dorsal views of embryos derived from germline clones homozygous for *l(1)dsh* at 9 and 13 hr. Note the absence of segmentation (arrow in (B)) and the degeneration of the amnioserosa. Anterior is up in all figures. Abbreviations: Clypeolabrum (CL), amnioserosa (As), and posterior spiracles (Sp).

cuticle which originates from the posterior compartment of every segmental unit we analyzed the *engrailed* expression in these embryos with an antibody directed against the *engrailed* gene product (DiNardo *et al.*, 1985). The expression of *engrailed* gene product in *dishevelled* embryos is normal at 5 hr of development (results not shown); but this pattern progressively degenerates. Figure 7B shows a 9 hr *l(1)dsh/Y* embryo derived from homozygous germline clones. At this time *engrailed* expression appears normal in the nervous system but is patchy and faint in the ectoderm (Fig. 7B compared to wild-type in 7A, see also DiNardo *et al.*, 1985 for a complete description of the distribution of *engrailed* protein in wild-type embryos). We attribute this pattern to the progressive degeneration of cells that belong to each posterior compartment. This result correlates with the pockets of cell death detectable at 6 hr of development and the absence of early defects in the nervous system. This result does not exclude the possiblity that cell death also occurs in the anterior compartment.

Comparison of the Maternal Effect of l(1)dsh with Other Segment Polarity Loci

Because of the discovery that *l(1)dsh* belongs to the class of segment polarity mutations, we compared it to the segment polarity embryonic phenotype exhibited by mutations at five other loci. In Table 1 are listed the mutations used in this study, their map location, and

FIG. 2. Scanning electron micrographs of embryos derived from germline clones of *l(1)dishevelled*. In (A–C) are shown three wild-type embryos at 7, 9, and 11 hr of development, respectively. (D–F) are shown similar stages of embryos derived from germline clones homozygous for *l(1)dsh*. These *l(1)dsh* embryos lack head and abdominal segments and the tracheal pits fuse laterally with each other (full arrows in (E) and (F)). A structure that resembles the maxillary appendage is indicated by an open arrow in (E). Anterior is up in all figures and ventral is on the left. Abbreviations: Mandibulary (Md), maxillary (Mx), and labial (Lb) segments; tracheal pits (Tp), salivary glands invagination (Sg), posterior spiracles (Sp), amnioserosa (As). P refers to a parasegment, S to a segment, T to the thoracic, and A to the abdominal region.

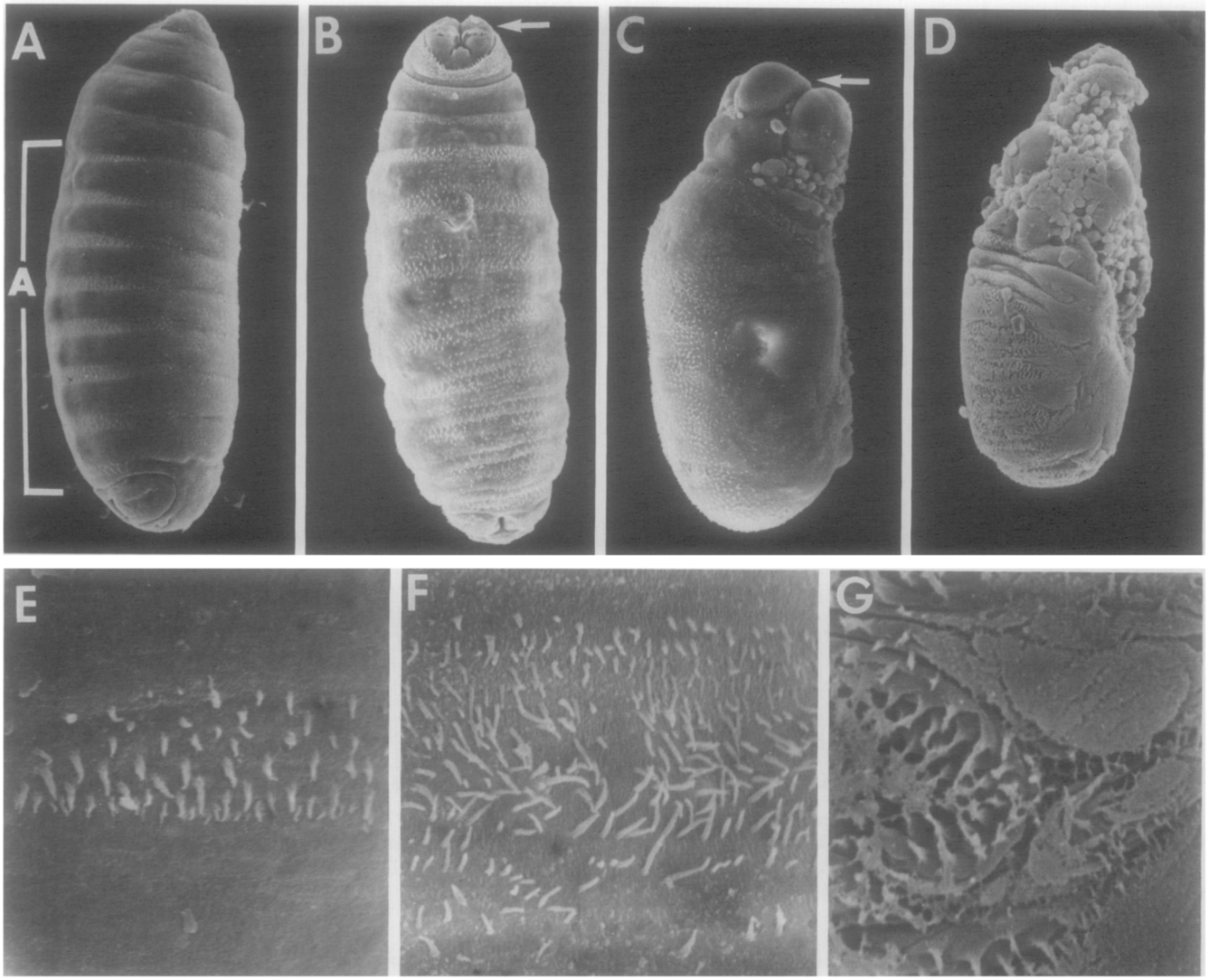

FIG. 4. Scanning electron micrographs of the late phenotype of segment polarity loci. (A) Is a lateral view of a wild-type embryo at 20 hr. Note the ventral denticle belts of the abdominal region (A) and the involuted head. (B) Is a ventral view of an *l(1)armadillo* embryo. The ventral denticle belts exhibit the reverse polarity phenotype. Segmentation is visible. In this embryo the head is not completely involuted (arrow). (C) and (D) are *dishevelled* embryos at 19 and 24 hr, respectively. Note the absence of segmentation and the extrusion of head (arrow in (C)) and gut structures. Details of the ventral denticle in (E) wild type, (F) *l(2)gooseberry*, and (G) *l(1)dishevelled* are shown. The reverse polarity of the setae is clearly visible in (F) and (G).

their origins. Four of these loci are embryonic lethal loci, *l(1)armadilo*, (*l(1)arm*, Wieschaus *et al.*, 1984), *l(2)wingless*, (*l(2)wg*); *l(2)gooseberry*,(*l(2)gsb*); and, *l(3)-hedgehog* (*l(3)hh*) (Nusslein-Volhard and Wieschaus, 1980; Nusslein-Volhard *et al.*, 1984; Jurgens *et al.*, 1984). The fifth locus is a late zygotic lethal, *l(1)fused*, (*l(1)fu*) (see below).

The Segment Polarity Phenotype of wingless is Similar to the Maternal Effect of l(1)dsh

Embryos homozygous for *l(2)wg* exhibit an extreme segment polarity phenotype. They lack head cuticle and there are no or very rudimentary filzkorper (Fig. 1E). The early development of *l(2)wg* embryos (Fig. 8) is comparable to *l(1)dsh* embryos derived from homozygous germline clones. A similar pattern of cell death and lack of parasegmental and segmental boundaries is observed. *l(2)wg* embryos differ from *dishevelled* embryos in that they differentiate more head segments (Fig. 8). The expression of *engrailed* in *l(2)wg* embryos is similar to the pattern observed in *dishevelled* embryos (result not shown; S. DiNardo, personal communication).

The Segment Polarity Phenotype of fused, armadillo, gooseberry, and hedgehog Is Different from the Phenotype of dishevelled and wingless

We prepared germline clones of the two lethal alleles of *fused* as well as *fs(1)fu*^{2P} (Tables 1, 3), since the latter

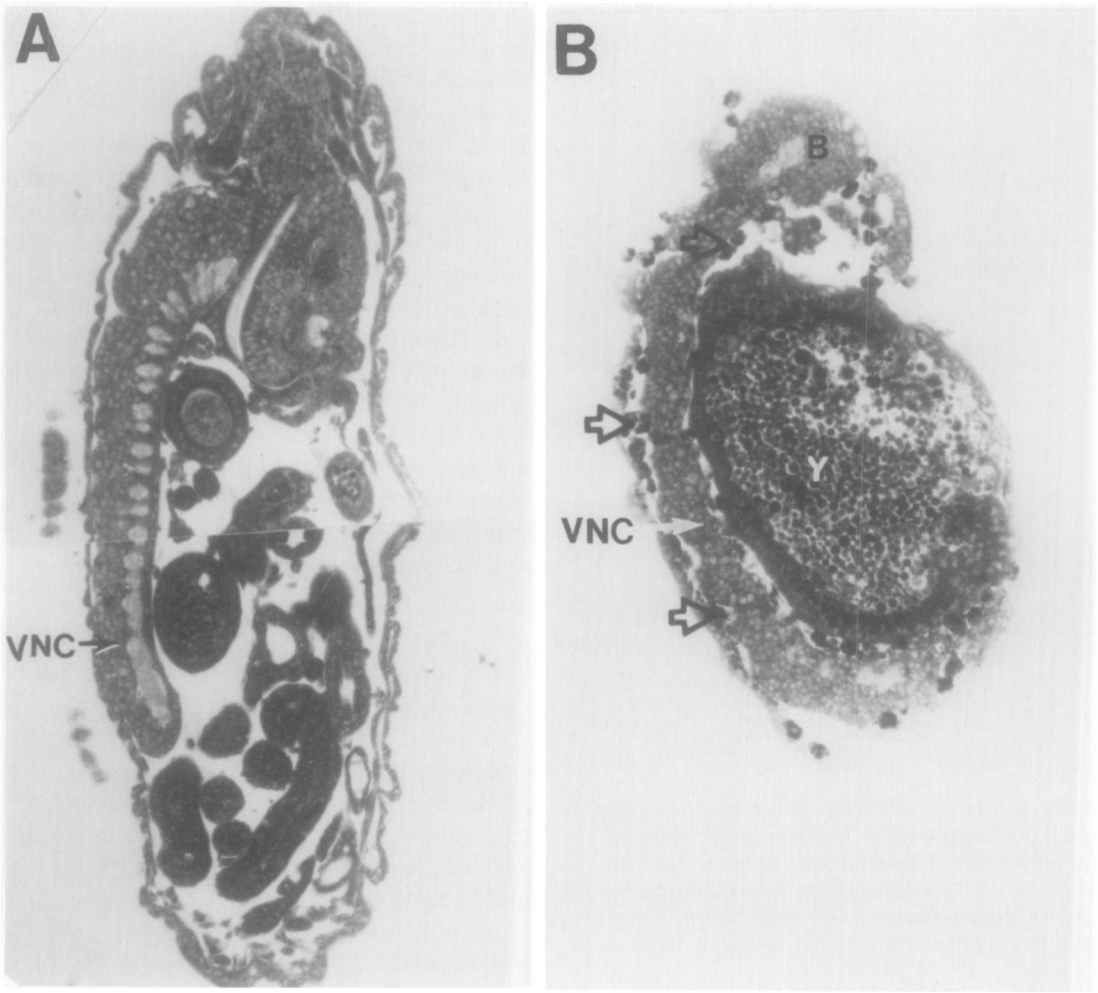

FIG. 5. Development of internal structures in embryos derived from germline clones homozygous for *l(1)dishevelled.* (A) and (B) represent longitudinal sections of wild-type and germline clone derived *l(1)dishevelled* embryos at 22 hr, respectively. Note in (B) the extensive cell death in the mesoderm (indicated by open arrows). Anterior is up and ventral is on the left. Abbreviations: Yolk (Y), brain (B), ventral nerve cord (VNC).

allele exhibits poor viability in homozygous females. In all three cases a similar rescuable, maternal effect phenotype was observed (Table 1). About 15 to 25% of the eggs derived from these germline clones arrest at early cleavage stages. These eggs because of their early mitotic arrest do not develop and have been scored as unfertilized eggs (Table 3). Among the remaining eggs the maternal effect is paternally rescuable; although this rescue is not fully penetrant (Table 3). In the case of flies possessing homozygous germline clones for the *l(1)fu*^{1PP7} allele, 34% of the developing embryos hatch, but only 10% of these become adults and most of these exhibit defects in thoracic or abdominal segmentation. With the *l(1)fu*^{9P2} allele, 31.5% of the embryos hatch and a larger fraction (about 90%) become adults. Interestingly, very few of these adults exhibit morphological defects.

Most of the eggs that complete blastoderm formation undergo gastrulation movements normally. At around 6 to 7 hr of development, patterns of cell death are detected that are similar to, but less extreme than, those observed in *l(1)dsh* embryos derived from germline clones (see also Martinez-Arias, 1985). Also, at this time *fused* embryos undergo correct segmentation. Occasionally, we observe the absence of segment borders in the posteriormost abdominal region at 8 hr of development (results not shown). Internally, most structures appear to develop normally, although breaks in the ventral nerve cord can be detected at 8–9 hr. When we examine the cuticle of embryos produced by homozygous *fused* alleles, two classes of embryos are found: (1) embryos that exhibit a strong segment polarity phenotype (Fig. 1C); and (2) embryos that show a weaker phenotype (Fig. 1B). By using the embryonic cuticle marker *yellow* (*y*), we have determined that embryos with the more extreme phenotype derive from eggs that have not received the *fu*$^{+}$ gene from the sperm (*y l(1)fu*/*Y*). Similarly, embryos

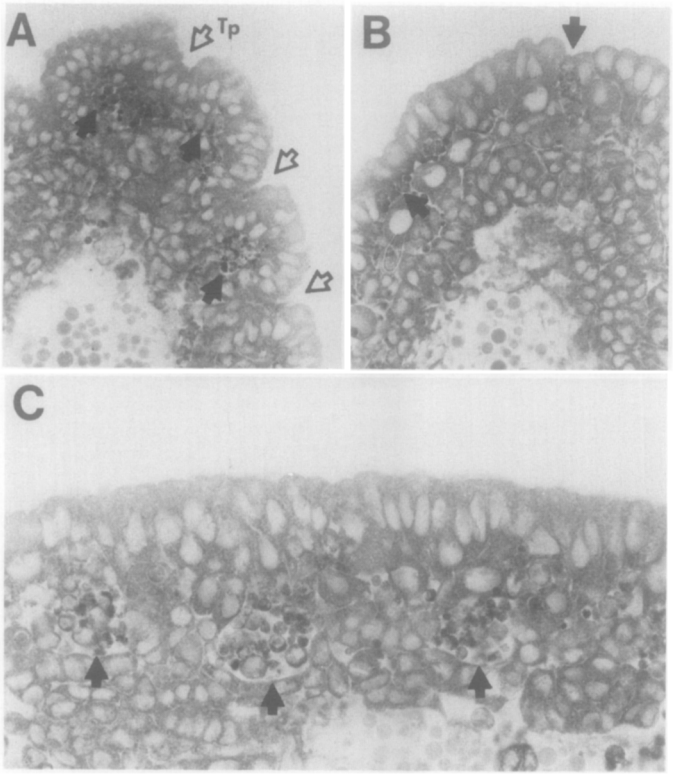

FIG. 6. Cell death associated with embryos derived from germline clones homozygous for *l(1)dishevelled*. (A) Is a section through the tracheal pits (Tp, indicated by open arrows) of a germline clone derived *l(1)dishevelled* embryos. (B) Cell death can be detected in the ventral ectoderm. (C) Shows three pockets of cell death below the ventral ectoderm. Cell deaths are indicated by full arrows in all figures.

with the weaker phenotype are paternally rescued embryos (*y l(1)fu/+*). *l(1)fu/Y* embryos derived from germline clones are missing the Keilin's organ but possess antennal and maxillary sense organs and normal posterior spiracles.

Homozygous *fs(1)fu* females are associated with an ovarian tumor phenotype (King, 1970) that increases with age. We also found ovarian tumors in flies carrying homozygous germline clones of $l(1)fu^{9P2}$, $l(1)fu^{1PP7}$, and $fs(1)fu^{2P}$, indicating that this phenotype is germline dependent. Transplantation of wild-type pole cell into *fused* embryos will be required to verify the purely germline function of *fused*.

l(1)arm, *l(2)gsb*, and *l(3)hh* embryos exhibit segment polarity phenotypes associated with variable head (Fig. 1D) and dorsal closure defects (the defects are especially notable in $l(1)arm^{XK22}$). These embryos always possess fully developed spiracles. Scanning electron micrographs indicate that segmentation occurs normally (Fig. 4B). Histological examination identified localized cell death similar to those observed in *fused* embryos. Therefore, the segment polarity defects in *l(1)arm*, *l(2)gsb*, and *l(3)hh* are similar to the maternal effect of *l(1)fu*. Since it is possible that the embryonic phenotype is influenced by the maternal contribution we prepared germline clones of the two mutations $l(1)arm^{XM19}$ and $l(1)arm^{XK22}$ (Table 1). Although no fecund females were obtained, 19 $l(1)arm^{XM19}+/+Fs(1)K1237$ and 15 $l(1)arm^{XK22}+/+Fs(1)K1237$ females were found to possess ovaries containing small eggs. These results indicate that arm^{+} is required during oogenesis. Similar results were recently obtained by Wieschaus and Noell (1986).

In conclusion, like *l(1)dsh*, *l(1)fu* exhibits a rescuable, maternal effect associated with a segment polarity phenotype. However, the maternal effect of *l(1)fu* differs from *l(1)dsh* in that segmentation is relatively normal, and the maternal effect is not fully rescuable. The segmentation defects observed in *dishevelled* and *wingless* embryos are not observed in *fused*, *gooseberry*, *armadillo*, and *hedgehog* embryos.

DISCUSSION

We have described the embryonic phenotypes associated with mutations at six segment polarity loci. Analysis of the development of early embryos by SEM, cuticle preparations, and histological techniques have allowed us to classify these loci into two groups: (1) the extreme segment polarity phenotype found in embryos derived from homozygous *l(1)dishevelled* germline clones and homozygous *l(2)wingless* embryos; (2) the weak segment polarity phenotype found in embryos mutant for *l(1)armadillo*, *l(2)gooseberry*, *l(3)hedgehog*, and embryos

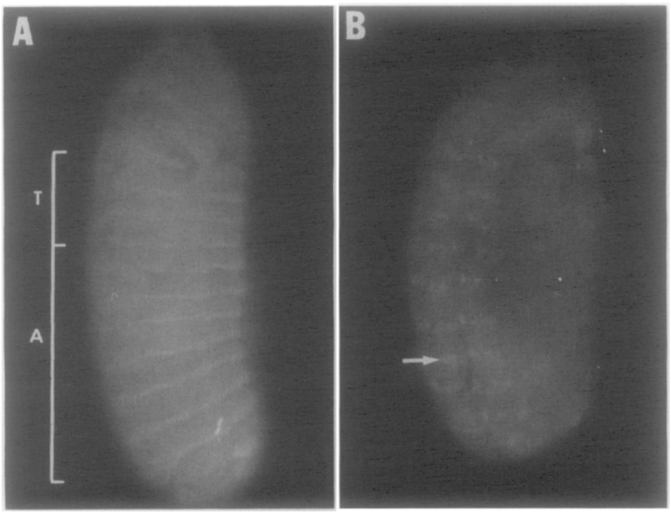

FIG. 7. Expression of engrailed in wild-type (A) and *dishevelled* (B) embryos. Note the regular striped pattern of engrailed expression in wild-type embryos (A). In *dishevelled* embryos a striped pattern of *engrailed* expression is visible in the nerve cord (arrow) and a patchy and faint pattern of expression of *engrailed* is detectable in the ectoderm (clearly visible laterally).

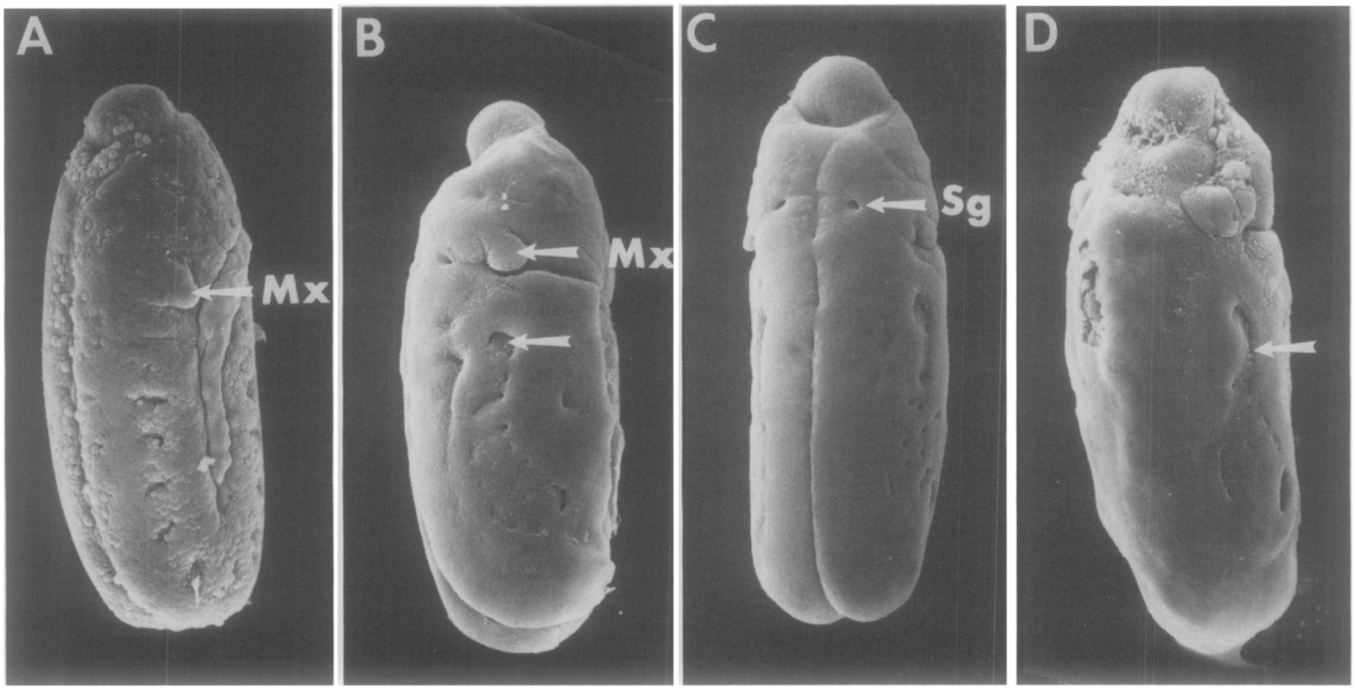

FIG. 8. Early development of *l(2)wingless* embryos. (A) Represents a *l(2)wg* embryo at 7 hr of development. Note the presence of a rudimentary head segment probably corresponding to the maxillary segment. (B) and (C) are lateral and ventral views of a 10 hr *l(2)wg* embryo. Note the absence of segmentation, the lateral fusion of tracheal pits (indicated by arrows), and the presence of the salivary gland invaginations. (D) Is a 18 hr unsegmented *l(2)wg* embryo. Note the absence of head involution. Abbreviations: Maxillary (Mx) segment and salivary gland invagination (Sg).

derived from homozygous *l(1)fused* germline clones. The principal phenotypic differences are observed at the time of tracheal pit formation. In extreme segment polarity embryos, no parasegmental or segmental boundaries are formed, resulting in unsegmented embryos. The phenotype is associated with segmentally spaced areas of hypodermal cell death located between the tracheal pits. Histological analysis reveals pockets of dead cells in the mesoderm which most probably originate in the overlying hypoderm and are rapidly removed to the interior. The loss of cells expressing the *engrailed* protein indicates that at least some, if not all, of the dead cells originate from the posterior compartment of each segmental unit. Additional markers will be required to determine whether all of the dead cells originate from the posterior compartment. In the weak segment polarity embryos, parasegmental as well as segmental boundaries are formed and cell death is less extensive. Because of the similarities between the patterns of cell death and the late cuticular patterns it is possible that all the segment polarity loci affect similar or related functions, but to different extents. The lack of parasegmental and segmental borders seen in the extreme segment polarity embryos may be correlated with the extent of cell death in the posterior compartments.

Do the phenotypic differences reflect different functions or different levels of gene activity? It is possible that stronger alleles of *l(2)gsb*, *l(1)arm*, *l(3)hh*, and *l(1)fu* might exhibit the extreme unsegmented embryonic phenotype. Alternatively, weaker alleles at the *l(1)dsh* or *l(2)wg* loci might allow parasegmental and segmental boundaries to form. So far we have not found examples of either alternatives, although many alleles at these loci have been examined. Since embryos homozygous for an embryonic lethal mutation are derived from heterozygous females, it is possible that maternally provided gene activity influences the embryonic phenotype. The analysis of the maternal contribution of *l(1)arm* supports such a hypothesis. *l(1)arm* embryos derived from heterozygous mothers exhibit a stronger phenotype than those derived from females homozygous for the wild-type allele (Wieschaus and Noell, 1986, unpublished observations), indicating that the maternal dosage of this gene is responsible for some of the phenotypic differences. Because $l(1)arm^{+}$ is also required for normal oogenesis, it is impossible to analyze the embryonic phenotype associated with lack of maternal and zygotic $l(1)arm^{+}$ gene activity. Pole cell transplantation experiments will be needed to understand the maternal contribution of the other segment polarity loci.

In Fig. 9 we provide a model to account for the defects observed in segment polarity mutations. In the strong

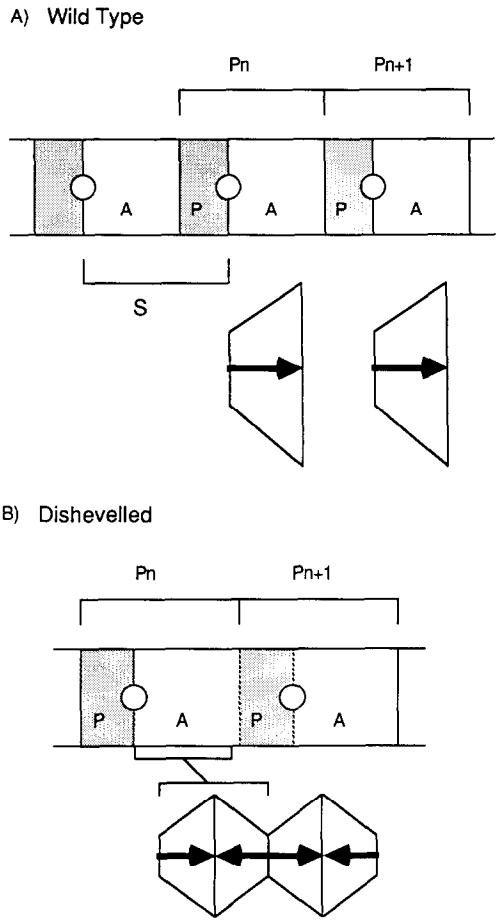

FIG. 9. Schematic drawing of the effect of *dishevelled*. Two abdominal parasegmental units (Pn and Pn+1) are related to compartments (anterior, A and posterior, P), segment (S) and tracheal pits (open circle at the segmental borders) in wild-type embryos. The orientation from anterior to posterior of the setae are schematized by black arrows and the trapezoides represent the shape of abdominal denticle belts. In *dishevelled* embryos we suggest that cell death in P compartment leads to the juxtaposition of cells from A compartments of different parasegmental units. This will generate two defects: (1) lack of formation of parasegmental and segmental grooves, and (2) reverse polarity phenotype (see Discussion).

segment polarity mutants, cell death, occurring in all posterior compartments, leads to the juxtaposition of cells from the anterior compartments of every segmental unit. A reverse polarity phenotype can be generated following a change in the positional values of the most posterior cells of the anterior compartment. These cells will change their positional values according to the route of intercalation of the shortest values (French *et al.*, 1976). The cells that give rise to the larval epidermis normally undergo only two rounds of cell division before differentiation (Szabad *et al.*, 1979; Campos-Ortega and Hartenstein, 1985). A number of possibilities occur following the massive cell death in the posterior compartment. Cells from the anterior compartment in an embryo with a segment polarity phenotype may undergo extra rounds of division. Alternatively, anterior cells may become reprogrammed without additional cell divisions.

The model proposed in Fig. 9 is supported by results of Martinez-Arias and Ingham (1985). Utilizing mutations of the *bithorax* complex, they found that the additional pattern elements in *gooseberry, hedgehog,* and *cubitus interruptus dominant* are derived from the anterior compartment of each segment. The results of Martinez-Arias and Ingham (1985) agree with results obtained from ablation experiments in *Oncopeltus*. Wright and Lawrence (1981) showed that the removal of a territory between two segments is followed by a reversal of polarity in the remaining part of the segment. The model proposed in Fig. 9 contrasts with the results of Gergen and Wieschaus (1986). In the case of mutations at two of the weak loci (*armadillo* and *fused*) (Gergen and Wieschaus, 1986), the mutations cause a cell autonomous phenotype. Thus, in gynandromorph mosaics, mutant cells in the posterior compartment express the segment polarity phenotype. Wild-type cells from the anterior compartment, detected by the "*shaven baby*" phenotype, do not contribute to the reverse polarity phenotype. Further analyses of this class of mutations is clearly necessary to reconcile the conflicting data.

Meinhardt (1986) has postulated the presence of a third component for each segment, termed S (separation) which is required for segmentation at the segment boundary between the anterior and posterior compartment. It is possible that the difference between the two groups of segment polarity mutations presented in this paper is due to the absence of the S component in *wingless* and germline clones of *dishevelled* and its presence in *armadillo, fused, goosberry,* and *hedgehog*. Unfortunately, at the present time we have no way to test this possibility.

Our analysis indicates that the segment polarity phenotype can be explained by a localized function of the gene products of segment polarity loci in the posterior compartment of each segment. Cells from posterior compartments may die because they lack a trophic factor or because they are not correctly determined. It is possible that these loci are involved in initiating and/or maintaining functions required to distinguish between cells from anterior and posterior compartments. Alternatively, these gene products might be involved in repressing genes normally only expressed in anterior compartments. Because *engrailed* is at first expressed normally in *dishevelled* and *wingless* embryos, we propose that the gene products encoded by these loci are not required to initiate but are necessary for the maintenance or expression of the determined state. In their absence these cells are unable to survive. Many

independent events might be involved in such processes and the segment polarity loci might act independently or coordinately. Because of their similar phenotypes it is possible that both *wingless* and *dishevelled* act together or in the same pathway. Analysis of the interaction of these two loci will provide additional information on these alternatives.

Developmental Pleiotropy

It is interesting that six of the seven segment polarity loci are associated with late imaginal functions. Somatic clonal analysis of *l(1)arm* (Wieschaus and Noell, 1986) and *l(3)hedgehog* (Mohler and Wieschaus, 1985) show that mitotic clones are associated with lethality. An allele of *wingless* affects mesothoracic and/or metathoracic imaginal disc development (Sharma and Chopra, 1976), although it has been argued that the defects in wing disc development caused by *wingless* alleles are determined very early in development, so that these defects may not require late imaginal functions for this gene. *Cubitus interuptus*, a fourth chromosome mutation that also displays the segment polarity phenotype (Nusslein-Volhard and Wieschaus, 1980) is associated with a dominant phenotype in which wings show interruptions of the L4 and L5 wing veins. Defects in wing size and the alula are also present (Lindsley and Zimm, 1985). The *fused* adult pehnotype results in "fusion of L3 and L4 wing veins, wings extended, ocelli defects, eyes reduced in size, anterior scutellar bristle usually missing, scutellum short" (Lindsley and Zimm, 1985). The *dishevelled* adult phenotype is associated wtih reversed polarity of some bristles and mirror image duplications of leg joints (Held *et al.*, 1986). These observations suggest that the dsh^+ gene product has analogous functions in embryonic development and in differentiation of some imaginal discs. As yet, no information is available for the imaginal function of *gooseberry*.

This developmental pleiotropy exhibited by these genes may not be uncommon during *Drosophila* development. For example, a set of loci identified as playing key roles during early embryogenesis are also associated with late functions. Among these are: *hairy* (Ingham *et al.*, 1985b), *engrailed* (Kornberg, 1981), *l(1)pole-hole* (Perrimon *et al.*, 1985), *l(1)hopscotch* (Perrimon and Mahowald, 1986b), (see review in Mahowald and Hardy, 1986; Perrimon and Mahowald, 1986a). These observations lead us to suggest that embryonic and imaginal development may share a subset of patterning genes. The analysis of late zygotic lethals with specific maternal effects on embryonic development may allow us to identify gene functions involved specifically in the patterning of imaginal discs.

We are grateful to: G. Lefevre, B. Geer, R. Voelker, D. Sponaugle, E. Wieschaus, the Bowling Green, and the Pasadena stock centers for providing stocks; S. DiNardo and P. O'Farrell for the gift of *engrailed* antibody; K. Maier for excellent technical assistance; P. Hardy and L. Perkins for comments on the manuscript. N. Perrimon is a Lucille P. Markey scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. This work was also supported by grants from the NIH (HD-17608) and the NSF PCM 84-09563.

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