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l(1)pole hole is required maternally for pattern formation in the terminal regions of the embryo

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Summary

Maternal expression of the *l(1)pole hole* (*l(1)ph*) gene product is required for the development of the *Drosophila* embryo. When maternal *l(1)ph*$^+$ activity is absent, alterations in the embryonic fate map occur as visualized by the expression of segmentation genes *fushi-tarazu* and *engrailed*. If both maternal and zygotic activity is absent, embryos degenerate around 7 h of development. If only maternal activity is missing, embryos complete embryogenesis and show deletions of both anterior and posterior structures. Anteriorly, structures originating from labral and acron head regions are missing. Posteriorly, abdominal segments A8, 9 and 10, the telson and the proctodeum are missing. Similar pattern deletions are observed in embryos derived from the terminal class of female sterile mutations. Thus, the maternal *l(1)ph*$^+$ gene product is required for the establishment of cell identities at the anterior and posterior poles of the *Drosophila* embryo.

Key words: pattern formation, maternal effect, embryogenesis, *Drosophila*.

Introduction

Pattern formation in the *Drosophila* embryo requires the regulation of maternal and zygotic gene activities to establish and maintain cellular identities within progressively smaller developmental fields. Initially, the interpretation of global embryonic polarity along the anterior–posterior and dorsal–ventral egg axes permits the establishment of specialized domains of zygotic gene expression (see reviews by Konrad *et al.* 1985; Mahowald & Hardy, 1985; Akam, 1987; Anderson, 1987; Nüsslein-Volhard *et al.* 1987; Perrimon & Mahowald, 1988; Ingham, 1988). It has been proposed that anterior–posterior polarity is determined by the generation of two gradients, each originating from opposite egg poles (Lehmann & Nüsslein-Volhard, 1986; Frohnhofer & Nüsslein-Volhard, 1986). The maternal *bicoid* gene product is responsible for the morphogenetic gradient originating at the anterior egg pole (Driever & Nüsslein-Volhard, 1988*a*,*b*). Maternal *nanos*$^+$ activity may serve as the posterior located morphogen (Nüsslein-Volhard *et al.* 1987; R. Lehmann, personal communication).

Independent of these gradients, activity encoded by the terminal class of maternal effect genes is required for the establishment of cell fates at the embryonic poles (Nüsslein-Volhard *et al.* 1982; Degelmann *et al.* 1986; Schupbach & Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987). Thus, in embryos lacking maternal terminal group function, both head and tail structures are absent. A similar phenotype is observed when embryos lack zygotic *tailless*$^+$ expression (Stecker *et al.* 1988). For head differentiation, maternal *bicoid*$^+$ function is also required. Embryos developing without *bicoid*$^+$ product have an anterior duplication of posterior pattern. Therefore, establishment of terminal cell fates requires maternal terminal group and zygotic *tailless* gene expression. The distinction between head and tail determinative pathways requires *bicoid*$^+$ function. Recent genetic experiments suggest that the maternal terminal genes act through the zygotic *tailless* gene (Klinger *et al.* 1988).

The terminal group of female sterile mutations contains five members including *fs(1)Nasrat* (Degelmann *et al.* 1986), *fs(1)pole hole*^{1901} (Perrimon *et al.* 1986), *fs(2)trunk*, *fs(2)torso* (Schupbach & Wieschaus, 1986), and *fs(3)torso-like* (Nüsslein-Volhard *et al.* 1987). All members of this class when defective in the female germline produce embryos showing deletions of anterior and posterior terminal cell fates (Mlodzik *et al.* 1987; Winslow *et al.* 1988). Presumably, these five maternal gene products function in a common pathway essential for the establishment of terminal cell identity. Another genetic locus, *l(1)pole hole* (*l(1)ph*), shows an equivalent maternal effect on embryonic development (Perrimon *et al.* 1985). Thus, it seems likely that the

l(1)ph gene product is also required for the expression of embryonic terminal cell fates. However, since *l(1)ph*$^+$ activity is also necessary at other stages of development, the diverse nature of its function has obscured its maternal role.

The *l(1)ph* gene was first identified as a zygotic lethal mutation causing death at the larval–pupal transition stage in *l(1)ph* hemi- or homozygous progeny derived from heterozygous females (Perrimon *et al.* 1985). Proliferation of imaginal cells fails to occur in these third instar larvae resulting in small, undifferentiated imaginal discs. Thus, zygotic synthesis of *l(1)ph*$^+$ product is essential for imaginal disc development, but it is not required for the growth of nondividing polyploid larval cells. Since all imaginal tissues are equally affected by *l(1)ph* mutations, it seems likely that expression of this gene plays a fundamental role in dividing larval cells.

The characterization of *l(1)ph* mutations using germline clonal analysis reveals an additional requirement for maternal *l(1)ph* expression during normal embryogenesis. Embryos derived from homozygous germline clones that have received a wild-type copy of the gene from their father phenotypically resemble embryos produced by the terminal class of female sterile mutations (Nüsslein-Volhard *et al.* 1982). They lack structures derived from abdominal segments 8, 9, 10, and the telson. These embryos will be referred to as the 'pole hole' embryos throughout the text. Those embryos derived from homozygous germline clones that have not received a wild-type copy of the gene from their father have a poorly differentiated exoskeleton. Throughout the text we will refer to these as 'null' embryos.

In this analysis, the maternal and embryonic requirements for *l(1)ph*$^+$ expression are described. We have analyzed the progression of development and the establishment of cellular identities in embryos derived from homozygous *l(1)ph* germline clones. Comparison of the maternal effect of *l(1)ph* with that produced by the terminal class of female sterile genes indicate that *l(1)pole hole* is a member of the terminal gene class.

Materials and methods

Strains

In this analysis, we used amorphic mutations at the *l(1)ph* locus including *l(1)ph*^{EA75}, *l(1)ph*^{C2Z2} and *l(1)ph*$^{11\text{-}29}$. These alleles of *l(1)ph* give similar phenotypes and throughout the text the alleles will not be noted. The chromosomes carrying *l(1)ph* mutations were maintained as *FM7c* stocks. The two *X*-linked maternal effect loci *fs(1)Nasrat*^{211} and *fs(1)pole hole*^{1901} are maintained in stocks balanced with *FM3* chromosomes. The autosomal maternal effect mutations: *fs(2)trunk*^{R153}, *cn bw sp/Cyo*, *fs(2)trunk*^{HH28}, *cn bw sp/Cyo*, *fs(2)torso*^{WK34}, *cn bw sp/Cyo*, *fs(2)torso*^{HH36}, *cn bw sp/Cyo* and *fs(3)torso-like*$^{035\text{-}6}$*/TM3, Sb* were obtained from T. Schupbach.

The *X*-linked dominant female sterile mutation *Fs(1)K1237* (Busson *et al.* 1983; Perrimon, 1984) is maintained as an attached-*X* stock: *C(1)DX, y f/Y* females crossed to *Fs(1)K1237, v*^{24}*/Y* males. The *engrailed/lacZ* (*en/lacZ*) strain was obtained from C. Hama and T. Kornberg; the *fushi-tarazu/lacZ* (*ftz/lacZ*) strain from Y. Hiromi and W. Gehring (1987); and the *even-skipped/lacZ* (*eve/lacZ*) strain from P. MacDonald (Lawrence *et al.* 1987).

Descriptions of balancer chromosomes and mutations can be found in Lindsley & Grell (1968). All experiments were performed at 25°C on standard *Drosophila* medium.

Clonal analysis

Germline clones of *l(1)ph* mutations were produced by using the dominant female sterile technique (Perrimon, 1984; Perrimon *et al.* 1984). Briefly, virgin females heterozygous for *l(1)ph/FM7c* were mated to *Fs(1)K1237 v*^{24}*/Y* males. At the end of the first larval instar stage, progeny were irradiated at a constant dose of 1000 rads (Torrex 120D X-ray machine; 100 kV, 5 mA, 3 mm aluminum filter). Mitotic recombination in the germline of *l(1)ph/Fs(1)K1237* females was detected by individual inspection of ovary development. The frequency of females carrying germline clones homozygous for *l(1)ph* was about 5%.

Embryo morphology

Cuticle preparations were prepared in Hoyers mountant as described by van der Meer (1977). Histological sections of embryos were prepared as described by Mahowald *et al.* (1979). Embryos were dehydrated and embedded in JB4 plastic (Polysciences). Serial 3 μm sections were cut using a Leitz 1516 microtome and stained with methylene blue. Slides were dried and mounted in Aquamount. Embryos were prepared for scanning electron microscopy as described by Turner & Mahowald (1976).

Observation of living embryos

Embryos were collected and observed through development as described by Wieschaus & Nüsslein-Volhard (1986).

Introduction of segmentation fusion genes

Females possessing homozygous germline clones for *l(1)ph* were crossed with males that carry either the *ftz/lacZ*, *en/lacZ* or *eve/lacZ* insertion. Similarly, in the case of the maternal effect lethal mutations, these transformants were introduced in mutant embryos by crossing flies homozygous for the maternal effect with males carrying the *lacZ* insertion. These promoter–fusion constructs show similar patterns of β-galactosidase expression in embryos as observed by antibody staining of the native protein for *ftz* (Hiromi & Gehring, 1987), *eve* (Frasch *et al.* 1987; Lawrence *et al.* 1987) and *en* (DiNardo *et al.* 1985; Klingensmith *et al.* 1989; C. Hama & T. Kornberg, personal communication).

Immunohistochemistry

Hoechst 33258 staining of embryos was performed as described by Wieschaus & Nüsslein-Volhard (1986). Immunohistochemistry was performed as described in Smouse *et al.* (1988) for tubulin using an antibody obtained from T. Karr; for segmentation genes using a mouse anti-β-galactosidase primary antibody from Promega-Biotec to detect β-galactosidase from *ftz/lacZ*, *en/lacZ*, and *eve/lacZ* genes; and for the *engrailed* protein using an antibody obtained from S. DiNardo and P. O'Farrell. To examine the central and peripheral nervous systems of mutant embryos, we used polyclonal antibody against horseradish peroxidase (anti-HRP), which labels all central and peripheral nervous system cell bodies and axons (Jan & Jan, 1982), and the SOX2 monoclonal antibody, which recognizes the cell bodies and axons of the entire PNS and subset of CNS neurons (Goodman *et al.* 1984). The anti-HRP antiserum was from Cappel.

Results

Early mitosis and cellular blastoderm formation is normal in both null and pole hole embryos

Nuclear cleavages and migration of nuclei to the egg cortex are normal in embryos derived from homozygous *l(1)ph* germline clones. As shown in Fig. 1, using Hoechst dye to stain nuclei (Fig. 1A) and a tubulin antibody to stain microtubules (Fig. 1B), embryos lacking maternal *l(1)ph*$^+$ gene product develop to the blastoderm stage with no morphological defects. Cell formation at the periphery is normal in these embryos except at the posterior pole where a hole in the blastoderm cell layer below the pole cells is observed. In a previous study (Perrimon *et al.* 1985), we indicated that null embryos failed to complete blastoderm cellularization. For these embryos, the distribution of nuclei around the egg cortex was also abnormal. Following the analysis of considerably more embryos produced by germline clones, we have found that the poorly cellularized blastoderm phenotype previously described actually depends upon the age of the mother rather than the genotype of the embryo. Among the embryos that have a normal morphology at the blastoderm stage, 50 % develop into pole hole embryos and 50 % into nulls. This is true for embryos derived from young mothers in which only 5 % of these eggs develop incomplete blastoderms, or embryos from old mothers in which a substantially greater number have early abnormalities. Thus the abnormal cellularization defect is related to the maternal rather than the zygotic genome.

The early pattern of gastrulation and morphogenetic movements

Gastrulation in wild-type embryos begins with the invagination of the midventral cells between 15 %–90 % egg length (0 % = posterior tip; 100 % = anterior tip) to form the embryonic mesoderm (Sonnenblick, 1950; Campos-Ortega & Hartenstein, 1985). Ventral furrow formation is normal in embryos derived from homozygous *l(1)ph* germline clones but extends posteriorly to approximately 5 % egg length. Both the cephalic furrow and the anterior midgut form correctly at 70 % and 85 % egg length, respectively, but formation of the posterior midgut invagination does not occur. In living embryos, germband extension is observed at 3·5 h of development for the majority of pole hole and null embryos. However, in 10 % of the embryos, a twist along the dorsal–ventral axis occurs at this time, producing corkscrew-shaped embryos (Fig. 3B). Relaxation of these twists is observed in the pole hole embryos prior to dorsal closure (Fig. 3C). Twisting occurs in both pole hole and null embryos (Fig. 3F). At 4·5 h of development (stage 10), the invagination of cells to form the stomodeum is observed for most embryos and by 7·5 h (stage 11) segmental grooves are present in all embryos. Therefore, from the time of fertilization until the completion of germband extension there are no external morphological differences between pole hole and null embryos.

Distinct maternal and zygotic requirements for l(1)ph$^+$ *activity*

Two phenotypic classes of embryos are observed by 8 h (stage 12) of development. At this time, approximately one-half the embryos undergo germband shortening and upon completing development show the terminal class phenotype (Figs 2D–F and 3C). For a small percentage of the embryos, the germband does not retract and U-shaped (Fig. 3D) pole hole embryos are formed.

Germband retraction does not occur in null embryos. Instead, development ceases at the elongated germband stage. As shown in Fig. 2G, the resulting embryos at 24 h consist of a ball of embryonic tissue at the anterior egg pole and an extruded mass of egg yolk at the posterior pole. Muscle contractions are observed in the anterior region of these living null embryos. However, the overall organization and differentiation of organ systems within the embryo is abnormal. This phenotype is the consequence of incomplete development and massive cell death (Fig. 3F). In the analysis of

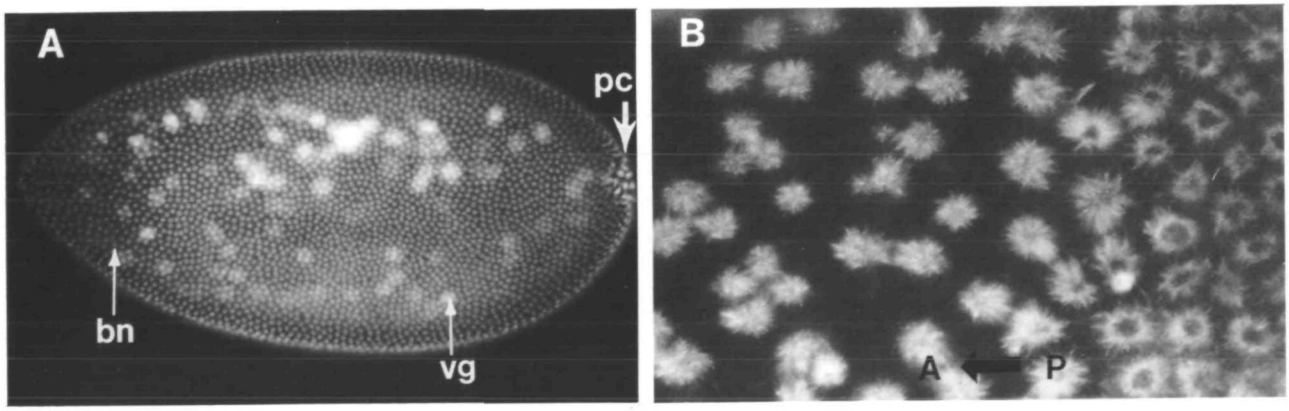

Fig. 1. Early mitosis and localization of nuclei in embryos derived from homozygous *l(1)ph* germline clones. (A) The distribution of blastoderm nuclei around the embryonic cortex, and the internal localization of yolk nuclei or vitellophages are normal in embryos derived from *l(1)ph* germline clones. At the posterior pole, the pole cells are visible. (B) A mitotic wave progressing normally from the posterior pole toward the middle of (see arrow) a *l(1)ph* germline clone embryo. *Abbreviations*: (bn) blastoderm nuclei; (pc) pole cells; (vg) vitellophages.

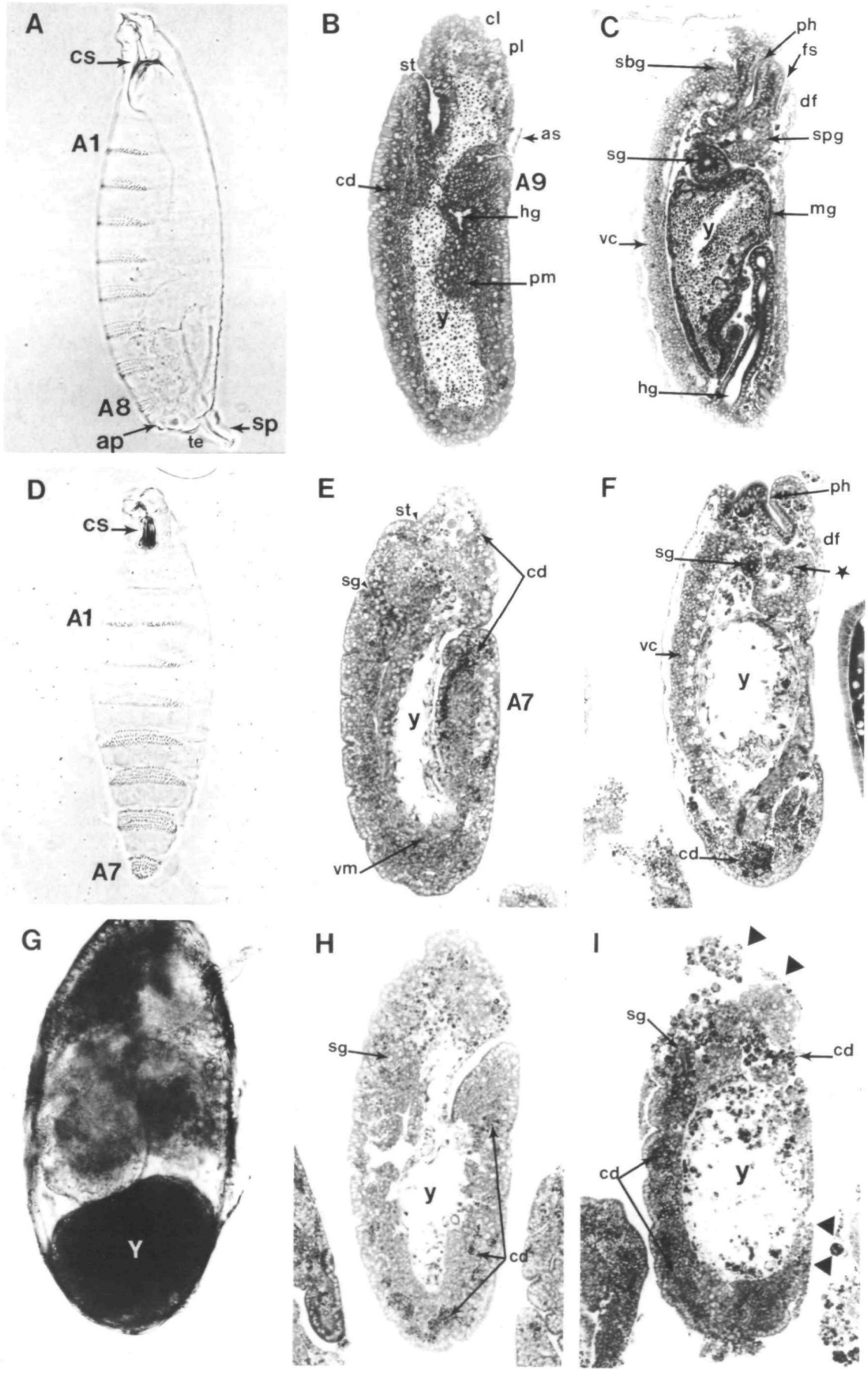
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Fig. 2. Phenotypic analysis of pole hole and null embryos. (A) Cuticular preparation of a wild-type embryo showing 8 abdominal segments (A1–A8), cephalopharyngeal skeleton, and terminal posterior structures including the anal plate, telson and spiracles. (B) A parasagittal section of a 6·5 h (stage 11) wild-type embryo. The germband is fully extended bringing A9 in close proximity with the procephalic lobe. Anteriorly, the clypeolabrum and stomodeum have formed. Note there are small pockets of cell death that occur at this stage during wild-type development. Yolk fills the internal region of the embryo. (C) A parasagittal section of a 12 h (stage 15) wild-type embryo. The frontal sac has formed as the result of the anterior progression of the dorsal fold. Yolk is enclosed by the midgut. (D) Cuticular preparation of a pole hole embryo showing the terminal class phenotype. Only 7 abdominal segments have formed (A1–A7) and the cephalopharyngeal skeleton is truncated. (E) A parasagittal section of a 7 h (stage 11) pole hole embryo. In the head and tail regions, a high level of cell death is observed. Derivatives of the proctodeum, including the hindgut and posterior midgut, are absent; however, the visceral mesoderm that normally joins these endodermal structures is present. Anteriorly, the stomodeum, which is slightly out of focus in this section, invaginates normally to form the pharynx and the oesophagus. Formation of the salivary glands appears normal. The terminal posterior segment is most likely A7. (F) A parasagittal section showing a 11 h (stage 14) pole hole embryo. The migration of the dorsal fold over the procephalic lobe to create the frontal sac will be complete at stage 15. The dorsal portion of the cephalopharyngeal skeleton is derived from the internalized procephalic lobe region and is abnormal in pole hole embryos. The cells that form the pharynx are also present, but differentiation of the cuticular posterior process of the pharynx is abnormal. The star (*) represents the anteriormost region of the central nervous system. Posteriorly, cell death is prominent, filling the space between the nerve cord and the epidermis. The periphery of the yolk sac has become cellularized. (G) A living null embryo after 20 h of development. All embryonic tissue is located at the anterior pole of the egg case while extruded yolk lies posteriorly. (H) A parasagittal section of a null embryo after 7 h of development (approximately stage 11). The germ band is extended but will not retract. The stomodeum (not shown here) and salivary glands have formed. At this time cell death is pronounced in the head and throughout the central region of the germ band. (I) A parasagittal section of a null embryo after 12 h. Massive cell death and loss of cells is apparent in the head and tail regions (triangles). Cell death is also found the internal region of the surviving thoracic and anterior abdominal segments. *Abbreviations*: (ap) anal plate; (as) amnioserosa; (cd) cell death; (cl) clypeolabrum; (cs) cephalopharyngeal skeleton; (df) dorsal fold; (fs) frontal sac; (h) hindgut; (mg) midgut; (pl) procephalic lobe; (ph) pharynx; (pm) posterior midgut; (sbg) suboesophageal ganglia; (sg) salivary gland; (sp) spiracles; (spg) supraoesophageal ganglia; (st) stomodeum; (te) telson; (vc) ventral nerve cord; (vm) visceral mesoderm; (y) yolk.

null tissue sections, cell death coincident with pycnotic nuclei is evident internally beginning at approximately 6·5 h. A tissue section of null embryo containing many pockets of cell death at 7 h is shown in Fig. 2H. After 12 h (Fig. 2I), the progression of cell death in the head and tail regions is so extreme that many cells are lost from the embryo (see triangles). In preparations of fixed embryos derived from *l(1)ph* germline clones, only fragments of null embryos containing the gnathal, thoracic and the anteriormost abdominal segments are recovered after 10 h. Cell death is also a consequence of wild-type development but is limited to small subepidermal clusters of necrotic cells that accumulate between 7 and 9 h of development (Fig. 2B). For pole hole embryos, many additional pycnotic nuclei are observed in the terminal embryonic regions (Fig. 2E and F).

Characterization of the fate map in pole hole and null embryos

Both maternal and zygotic gene products play a role in determining cellular identities at the time of blastoderm formation. In the mature embryo, alterations in head and tail regions are apparent in embryos lacking maternal $l(1)ph^+$ product. To determine whether the establishment of segmental identities in these regions depends upon functional maternal $l(1)ph^+$ activity, the expression patterns of the *fushi-tarazu* (*ftz*) and *engrailed* (*en*) segmentation genes were characterized in embryos derived from homozygous *l(1)ph* germline clones.

As shown in Fig. 4, at the blastoderm stage, instead of the seven alternating stripes of *ftz* expression observed in the wild-type embryo (Fig. 4A), only six regions of *ftz* activity were detected by β-galactosidase expression in *ftz*/lacZ embryos lacking maternal $l(1)ph^+$ function (Fig. 4B). For this embryo, the distance between the fifth and sixth stripe is greater than that of the normal embryo, and the sixth stripe is broader than the preceding anterior five stripes. Therefore, in the absence of maternal $l(1)ph^+$ activity, cell fates in the posterior region of the embryo are changed. A deletion of terminal identities is observed and cells forming in this region assume more anterior fates. This altered segmental pattern is observed for all embryos derived from homozygous *l(1)ph* germline clones and at this stage pole hole and null embryos are phenotypically equivalent.

When *engrailed* (*en*) expression is compared between embryos derived from homozygous *l(1)ph* germline clones, variation in the striping pattern is observed. Cells that express *en* label the posterior compartment of the segmental unit. In early wild-type gastrulating embryos there are 15 stripes of *en* expression (Fig. 5A). Along the longitudinal embryonic axis the labial, 3 thoracic and 9 abdominal segments show three nonstaining nuclei and one *en* nucleus per segment (DiNardo *et al.* 1985). In addition, approximately 11 nonstaining cells lie posterior to the 9th abdominal *en* stripe at the blastoderm stage. For embryos derived from homozygous *l(1)ph* germline clones, only 12 or 13 stripes of *en* expression form (Fig. 5B). When cells were counted along the longitudinal axes of *l(1)ph* germline clone embryos, variation in the number of cells per segment in the region of expanded segmental identities were observed between embryos. Fig. 5C2

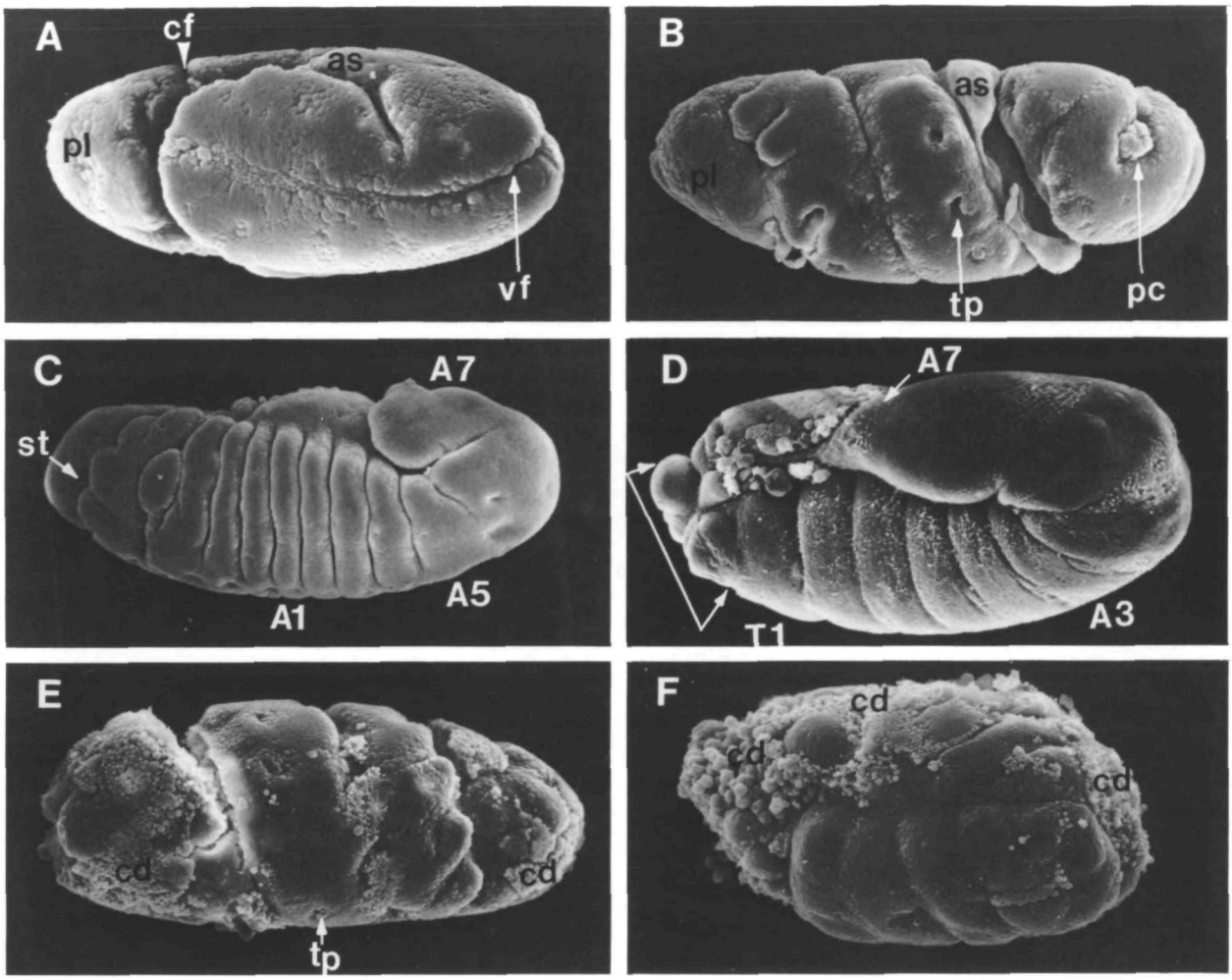

Fig. 3. Scanning electron micrograph analysis of pole hole and null embryos. (A and B) Embryos derived from *l(1)ph* germline clones at 6 and 7·5 h respectively. The distinction between pole hole and null embryos cannot be made based on external morphology at these times. The germband is extended in A and an example of a twisted embryo with external pole cells is shown in B. (C) An 11 h (stage 14) pole hole embryo showing seven abdominal segments, the germband is not fully retracted. (D) A U-shaped pole hole embryo at 20 h after cuticle formation. Arrows delimit the head region containing noninvoluted gnathal segments. (E) A twisted null embryo after 7 h. (F) A nontwisted null embryo after 15 h showing regions of massive cell death. *Abbreviations*: (as) amnioserosa; (cd) cell death; (cf) cephalic furrow; (pc) pole cells; (pl) procephalic lobe; (st) stomodeum; (tp) tracheal pit; (vf) ventral furrow.

and 5C3 depicts schematically the variation in *en* expression between two embryos lacking maternal *l(1)ph*$^+$ activity as compared to the wild-type expression pattern (Fig. 5C1).

Within a population of embryos derived from homozygous *l(1)ph* germline clones, many variations in segmental width for the region of expanded cell identities are observed. This variation is due to differences in cellular determination between embryos and not a secondary consequence of early cell death, since pycnotic nuclei are not observed until 6·5 h. For the 30 embryos derived from germline clones analyzed by counting the number of cells along the width of a segment, at 3–4 h the deleted pattern elements always included the *en* stripes 14–15 (posterior compartment of A8, and all of A9) and the posterior nonstaining region (A10, telson, and the endodermal derivatives of the proctodeum). Most often (60 %) these embryos were also missing the eighth abdominal segment and the posterior compartment of A7 (Fig. 5E). Larger deletions were observed in 10 % of the embryos. For the remaining embryos, 20 % showed expression of a 13th *en* stripe (Fig. 5B) and 10 % contained nonstaining cells (A8 anterior) after the 13th stripe. The segmental identities typically expanded to fill this deleted region of the fate map (approximately 20 %) were A5, A6 and A7. Occasionally, additional cells were observed in A3, and A4.

Characterization of the anterior defect in embryos lacking maternal l(1)ph$^+$ *activity*

The second region of the embryo affected by the loss of maternal *l(1)ph*$^+$ product is the head. In wild-type extended germband embryos, the *en* antibody labels nuclei in three ectodermal regions on either side of the head, forming a characteristic striping pattern (Fig. 5D;

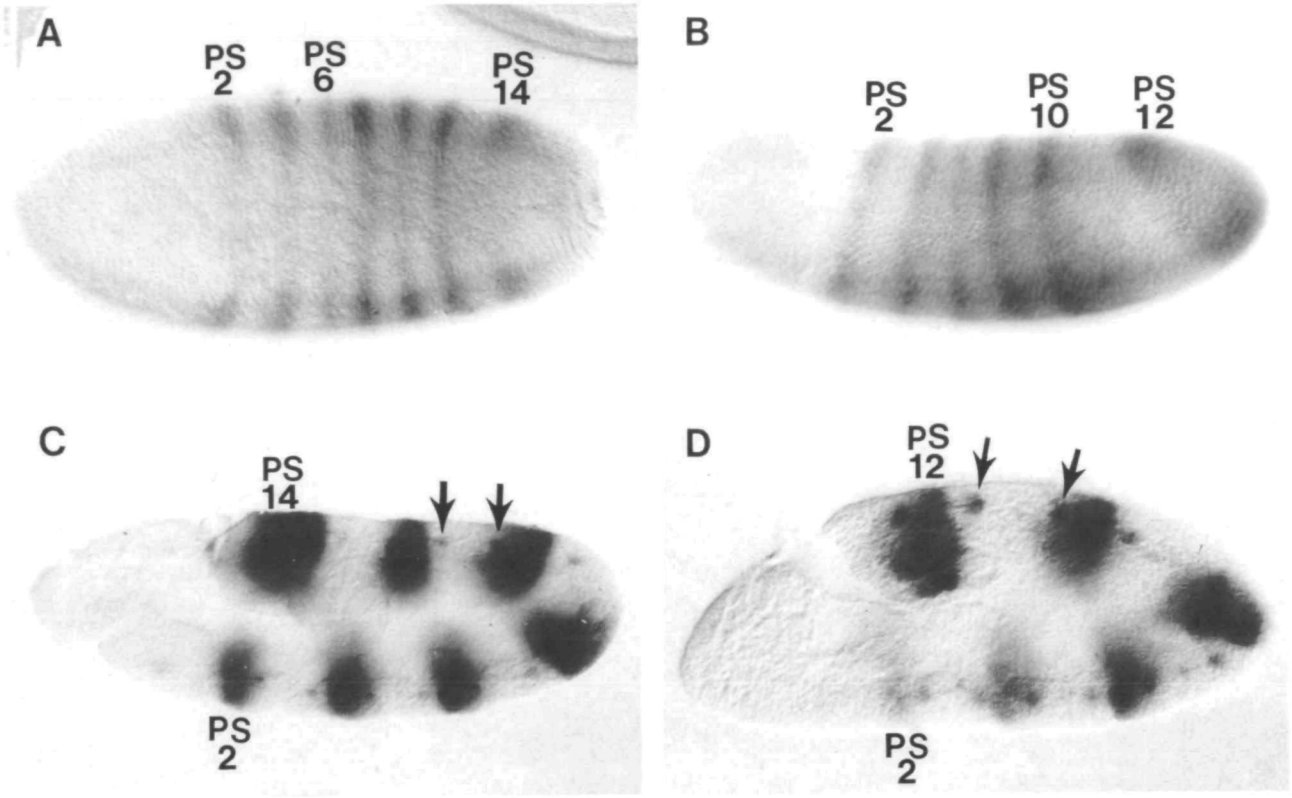

Fig. 4. Characterization of *ftz* expression in embryos derived from *l(1)ph* germline clones. The pattern of *ftz/lacZ* expression is used as a marker to characterize the embryonic fate map and to identify the MP2 neuroblasts of the central nervous system. (A) and (C) Wild-type embryos at the blastoderm (3 h) and extended germband stage (5·5 h), respectively, showing seven stripes of *ftz* expression. The center of each parasegmental stripe and nonstriped region defines the position where a segmental boundary will later form. At 5 h *ftz* expression is also detected in the MP2 neuroblasts (arrows) derived from the anterior compartment of each segment. (B) and (D) Embryos derived from *l(1)ph* germline clones showing only six regions of *ftz* expression. Note the expanded width of striped and nonstriped regions in the posterior of these embryos and the normal number of MP2 cells formed in the enlarged segments. *Abbreviations*: (PS) parasegment; (arrows) MP2 cells of the central nervous system.

also see DiNardo *et al.* 1985). A similar number and arrangement of *en* head stripes are present in embryos developing without maternal $l(1)ph^+$ activity (Fig. 5E). This region of *en* labeling does not correspond to the head region affected by lack of maternal $l(1)ph^+$ activity. To define the head defect and its relation to the embryonic fate map, the formation of cuticular head structures were analyzed in pole hole embryos. Also, the nervous systems of these embryos were examined using antibodies against horseradish peroxidase (HRP), which labels all central (CNS) and peripheral (PNS) nervous system cell bodies and axons (Jan & Jan, 1982), and SOX2, which recognizes the cell bodies and axons of the PNS and a subset of CNS neurons (Goodman *et al.* 1984). Based on the origin of the mutant structures identified, the regions of the head affected by the lack of maternal $l(1)ph^+$ product can be defined and correlated to the embryonic fate map.

In cuticular preparations of mature pole hole embryos, several defects in the head skeleton are apparent. The cephalopharyngeal apparatus (Fig. 6A) is formed by the involution of head segments and the internalmost elements are derived from regions of the head distal to the anterior pole (Campos-Ortega & Hartenstein, 1985; Jurgens *et al.* 1986). This apparatus is secreted by cells lining the atrium, pharynx and frontal sac. The truncated apparatus observed in pole hole embryos is thickened and collapsed (Fig. 6B). In detail, the dorsal bridge is absent and the lateral wall of the dorsal process reduced. In addition, the ventral process of the pharynx is abnormal. These structures of the mouth skeleton arise from the acron region of the fate map (Jurgens *et al.* 1986). The medial tooth derived from the labral head segment is also absent. Thus, the labrum and acron are two head regions affected by the absence of maternal $l(1)ph^+$ product. The labrum occupies an anterior–dorsal position on the embryonic fate map while the adjacent acron region extends posteriorly and laterally; together these two regions define approximately the dorsal half of the procephalic region (Jurgens *et al.* 1986).

When the anterior nervous system in a pole hole embryo is examined, the structures affected by the lack of maternal $l(1)ph^+$ function also originate from the acron and labrum regions. As shown in Fig. 7, much of the brain (supraoesophageal ganglia) is missing in the

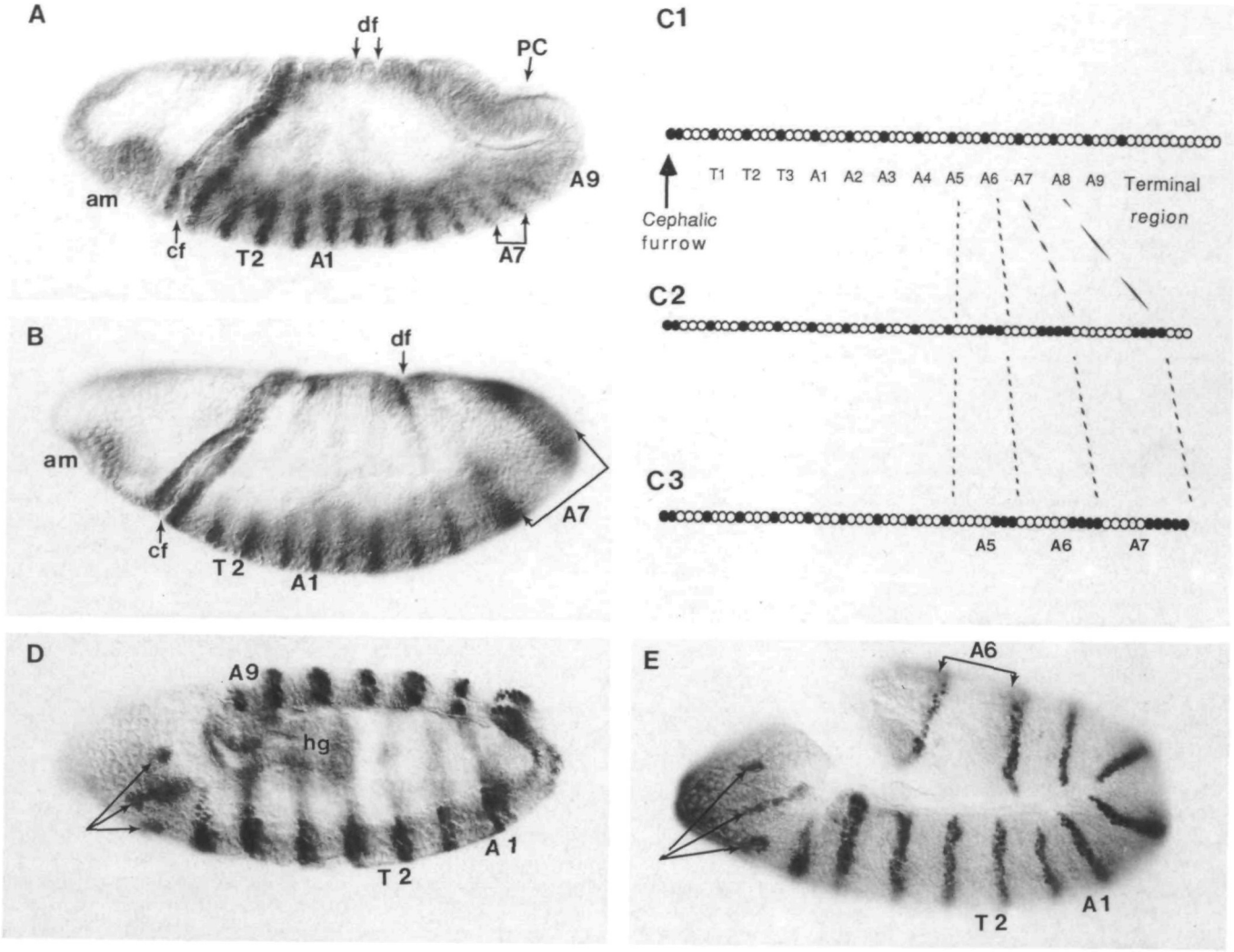

Fig. 5. *Engrailed* protein expression in embryos lacking maternal *l(1)ph* gene product. (A) An early gastrulating (3·5 h) wild-type embryo showing 15 stripes (the 2nd stripe lies in the groove of the cephalic furrow) of *en* expression. (B) A 3·5 h embryo derived from a *l(1)ph* germline clone with only 13 regions of *en* expression. The cell fates deleted include A8, A9 (interstripe and stripe 14 and 15), A10 and the telson (posterior nonstriped region). (C1, C2, and C3) A representation of blastoderm cells (circles) beginning at the cephalic furrow and extending along the longitudinal axis to the posterior tip of the embryo; the cells whose nuclei contain the *en* protein are filled in. (C1) For the wild-type embryo each segment is typically composed of three nonstaining (anterior compartment) and one *en* cell (posterior compartment). (C2 and C3) For embryos derived from *l(1)ph* germline clones the pattern of *en* expression in the posterior region is variable. Both the extent of segmental deletions and expansions vary between embryos. (D and E) A wild-type and *l(1)ph* germline clone embryo at 5·5–6 h (stage 11) with three head stripes of *en* expression (arrows). *Abbreviations:* (A) abdominal segment; (am) anterior midgut; (cf) cephalic furrow; (df) dorsal fold; (hg) hindgut; (pc) pole cells; (T) thoracic segment.

16 h pole hole embryo (Fig. 7D) when compared to the wild-type (Fig. 7B). The brain lobes develop from neuroblasts originating from a medial–lateral position on either side of the acron. Another distinguishable structure derived from the acron are the optic lobes. The optic lobes arise as a single placode at a dorsal–posterior position of the acron that invaginates at 7 h (stage 11) to join the developing supraoesophageal ganglia on its ventral side. The trajectory of Bolwig's nerve serves as a good marker for the localization of the optic lobe anlagen in wild-type and pole hole embryos (Steller *et al.* 1987). Fig. 8A shows the extension of Bolwig's nerve (arrows) in a wild-type embryo from cell bodies forming the larval photoreceptor cluster into the optic lobe region of the supraoesophageal ganglia. For pole hole embryos, however, the axons of Bolwig's nerve project to a dorsal–posterior position of the procephalic lobe (Fig. 8B and 8C). In wild-type embryos, this region would normally give rise to the optic anlagen. Wandering of Bolwig's nerve beyond this region is observed in some pole hole embryos at later stages of embryogenesis (data not shown). Thus, the brain and optic lobe anlagen, derivatives of the acron, are altered by the maternal effect of *l(1)ph*.

For the peripheral nervous system, the labral sense organ, originating from the labrum, is absent in pole

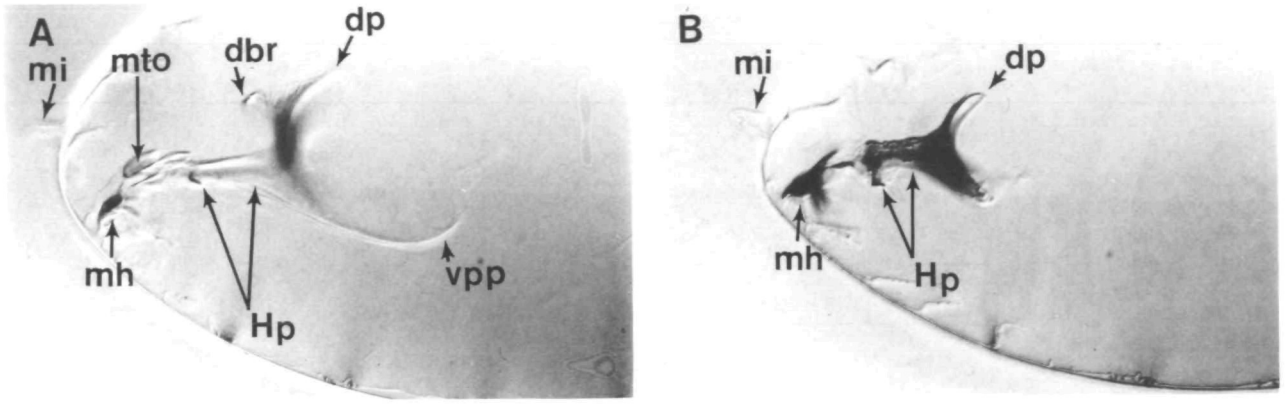

Fig. 6. Head skeletal defects in pole hole embryos. Organization of the cephalopharyngeal skeleton in a wild-type (A) and a pole hole (B) embryo. The head skeleton is formed by the involution of the dorsal procephalic and labral regions to form the frontal sac; and the clypeolabrum and gnathal segments to form the atrium and pharynx of the foregut. In pole hole embryos the medial tooth, dorsal bridge, and ventral posterior process of the pharynx are absent. The H-piece appears shortened and the dorsal process is abnormal. The mouth hooks of maxillary origin are normal in pole hole embryos. Note in this preparation, both embryos are encased in their outer egg membrane and the micropyle is visible. *Abbreviations*: (dp) dorsal process; (Hp) H-piece; (mh) mouth hooks; (mi) micropyle; (mto) medial tooth; (vpp) ventral posterior process.

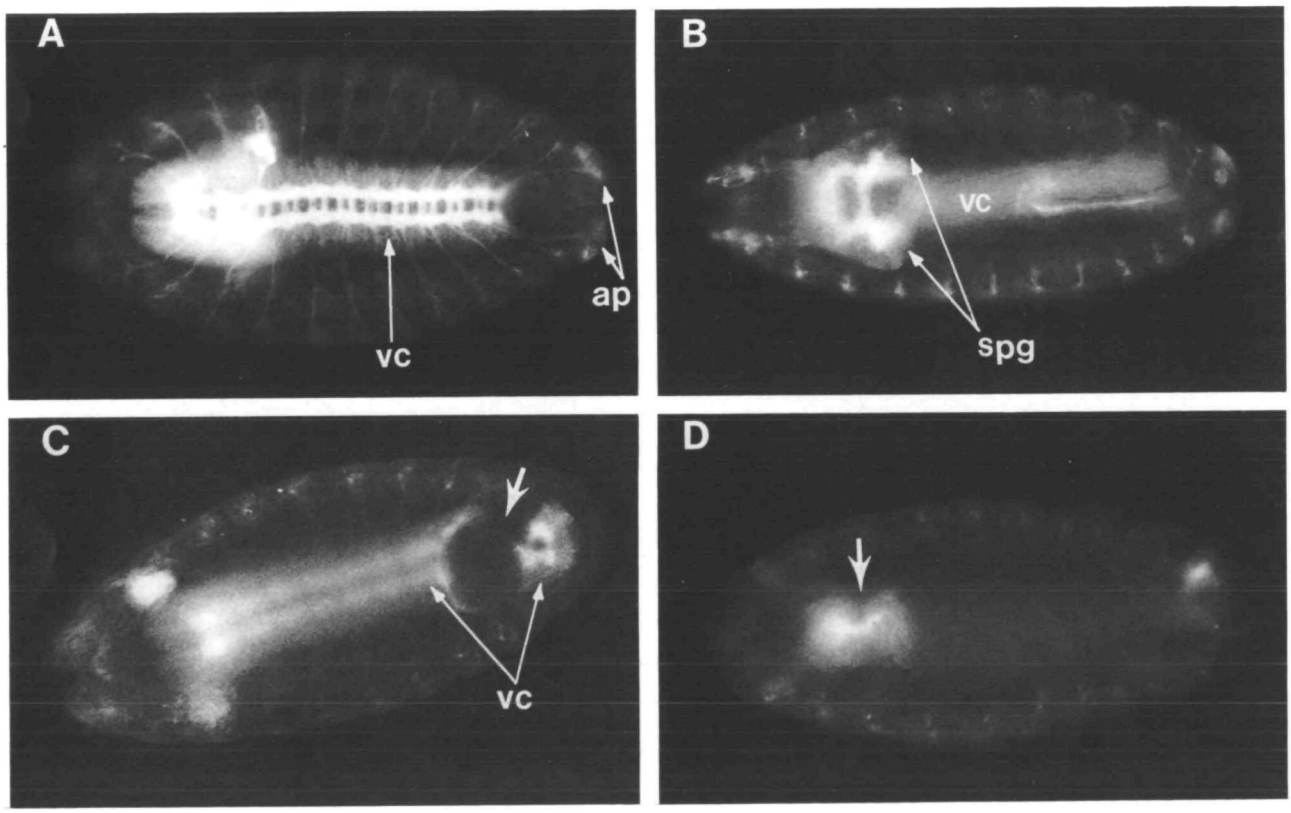

Fig. 7. Pattern of the central nervous system in pole hole embryos. HRP antibody staining of the nervous system at 16 h in wild-type (A and B) and pole hole (C and D) embryos. A ventral optical section showing the ventral nerve cord in a wild-type (A) and pole hole embryo (C). Note the posterior deformation of the CNS ladder (arrow) observed after nerve cord condensation for many pole hole embryos. The anal pads stain with the HRP antibody in wild-type embryos. Dorsal optical sections of the anteriormost region of the CNS in wild-type (B) and pole hole (D) embryos. For the wild-type, the brain lobes or supraoesophageal ganglia are evident, but are absent in pole hole embryos (arrow). *Abbreviations*: (ap) anal pads; (spg) supraoesophageal ganglia; (vc) ventral nerve cord.

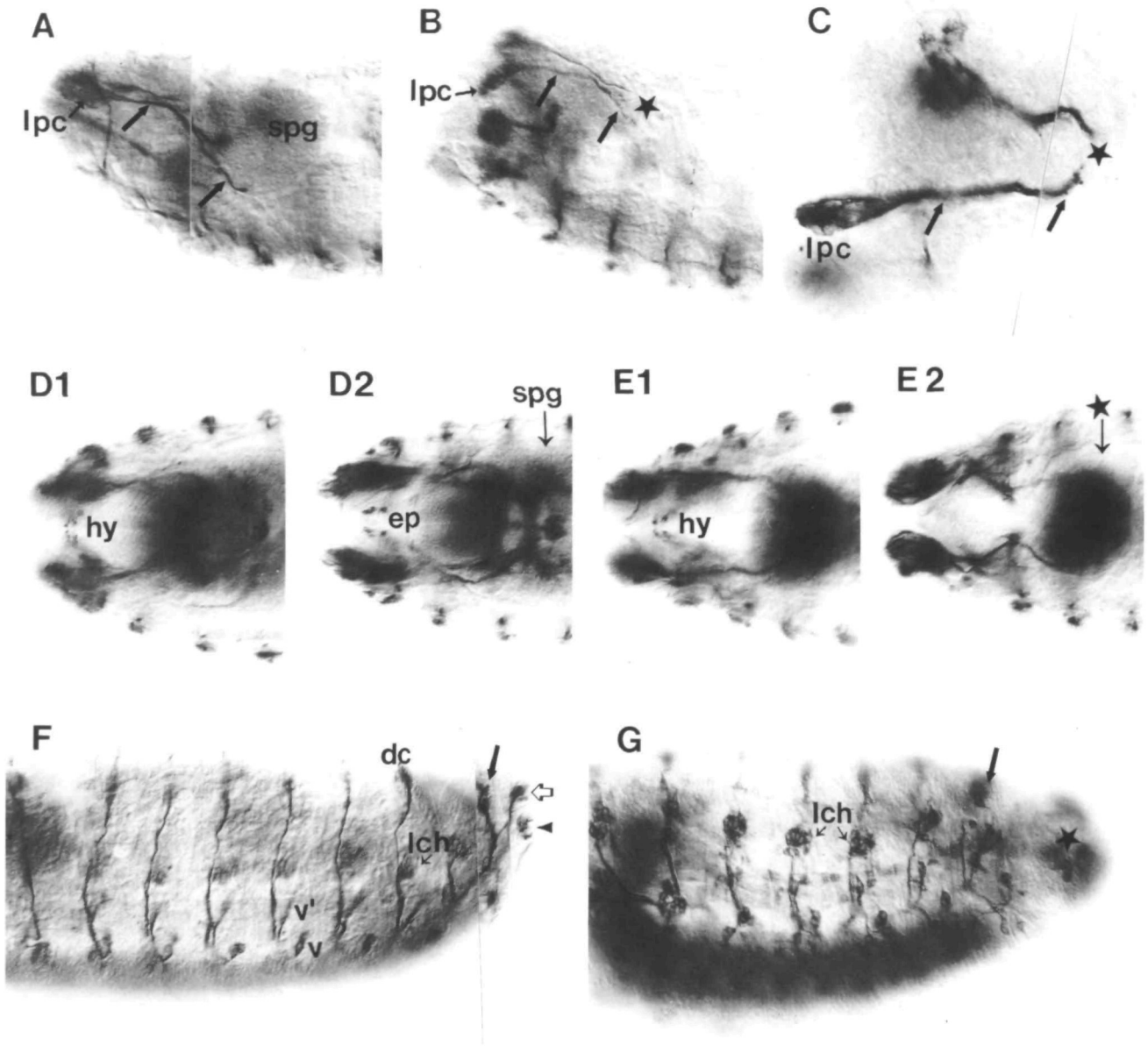

Fig. 8. Pattern of the peripheral nervous system in pole hole embryos. Embryos are stained with the SOX2 antibody. (A) Lateral view of a 16 h wild-type embryo showing the trajectory of Bolwig's nerve (arrows) into the optic lobe region of the supraoesophageal ganglia. The axons of Bolwig's nerve (arrows) originate from the larval photoreceptor cluster and terminate on the optic lobe located on the ventral surface of the brain lobes. (B) The trajectory of Bolwig's nerve (arrows) in a 14 h pole hole embryo. The brain lobes are missing in these embryos and Bolwig's nerve projects to a dorsoposterior region of the procephalic epidermis that normally gives rise to the optic lobes (*). (C) Localization of larval photoreceptor cells and Bolwig's nerve (arrows) in pole hole embryos at 11 h. (D1 through E2) Horizontal views of 17 h embryos showing the positions of two cephalopharyngeal sense organs: the hypophysis and epiphysis. For wild-type embryos, the hypophysis (D1) lies in an optical section ventral to the epiphysis (D2). For the pole hole embryo, the hypophysis is present (E1), but in more dorsal optical sections the epiphysis (E2) is missing; note the absence of the supraoesophageal ganglia (*). (F and G) Lateral views of the abdominal PNS in wild-type and pole hole embryos, respectively (see also Ghysen *et al.* 1986). A characteristic pattern of sensory cells are present for each segment in wild-type embryos; these include cells of the dorsal cluster, lateral chordotonals, and ventral cell clusters. For pole hole embryos, no duplications of sensory organs are observed in the regions of expanded segmental identities. The posteriormost complete segment often shows a dorsolateral displacement of lateral chordotonals (arrow) and a fusion of cells in the dorsal cluster (not shown here). In the terminal region, the specialized sensory organs of A8 and A9 (see arrows in F) are absent in pole hole embryos (*). *Abbreviations*: (dc) dorsal cluster; (ep) epiphysis; (hy) hypophysis; (lch) lateral chordotonals; (lpc) larval photoreceptor cells; (spg) supraoesophageal ganglia; (v and v') ventral cell clusters.

hole embryos. The wild-type head skeleton contains two sense organs, the hypophysis derived from the labial head segment (Fig. 8D1) and the labral-derived sense organ, the epiphysis (Fig. 8D2). For embryos lacking maternal *l(1)ph*$^+$ function, the hypophysis is present (Fig. 8E1); however, in more dorsal optical sections, the labral sense organ is missing (Fig. 8E2). Since the other sensory elements derived from the remaining head segments develop normally in pole hole embryos, the labrum and acron are the two areas of embryonic fate map in the head altered by the maternal effect of *l(1)ph*.

Consequences of the expansion of the posterior fate map in pole hole embryos

Since there are more ectodermal cells per segment in the tail region of 3 h pole hole embryos, this change may alter the subsequent pattern of the central nervous system derived from these enlarged segments. Briefly, neuroblasts leave the ectodermal layer at 4–6 h and are localized between the mesoderm and ectoderm. They undergo a series of divisions and then progeny neurons extend their axons in a specific manner to form the wild-type CNS ladder structure observed in Fig. 7A. The identification of the MP2 neuroblasts (Doe *et al.* 1988) serve as a good marker to analyze the pattern of segmental neuronal determination in pole hole embryos. First, the MP2 cells express the *ftz* gene and by 5 h these cells are prominently stained in wild-type *ftz/lacZ* embryos (Fig. 4C). Secondly, since one set of MP2 cells arises from the anterior compartment of each abdominal segment, an alteration in the specification of MP2 neuroblasts in the expanded segmental fields would be easily detected. As shown in Fig. 4D for a pole hole embryo there is no duplication of MP2 neuroblasts in the region of expanded cell identities. It appears that the integrity of a single segment (and/or parasegment) is maintained in pole hole embryos and in the presence of excess ectodermal cells one set of MP2 neuroblasts are formed per segment. A deformation of the CNS ladder is observed for many pole hole embryos after nerve cord condensation in the region of expanded segmental identities (Fig. 7C). Usually, the anterior and posterior commissures are normal in the last complete terminal segment but breaks in the longitudinal axis of the CNS are observed (arrow). Perhaps, the presence of large numbers of dying cells interfere with the formation of axonal tracts in these regions. In the posteriormost region of pole hole embryos, ill-defined bundles of HRP staining cells are detected.

For the peripheral nervous system of pole hole embryos (Fig. 8G), the wild-type number of sensory cells per abdominal segment are observed in the enlarged segments but, in some cases, these elements are displaced laterally. In addition, fusion of the dorsal cell cluster is observed along the dorsal midline and the positions of the lateral chordotonal cells are shifted dorsally (Fig. 8G, arrow) in the posteriormost complete segment of pole hole embryos. For the terminalmost region, cells staining with SOX2 are observed (*), but these cells cannot be identified morphologically and they form several fused bundles at later stages of development.

Therefore, within the region of expanded segmental identities, the nervous system of pole hole embryos develops much like the more anterior unaffected segments. In the last incomplete segment at the posterior pole, the central and peripheral nervous systems are abnormal. In null embryos the development of the nervous system is severely affected by the lack of *l(1)ph*$^+$ activity and only isolated clusters of HRP and SOX2 staining cells are detected; there is no organization of these cells to produce a ladder-like structure or to differentiate into morphologically identifiable sensory cells.

Comparison between the maternal effects of l(1)ph *and the terminal class of female sterile mutations*

Table 1 lists the terminal class of female sterile mutations and the phenotypic changes associated with the lack of these maternal products during embryonic development (Degelmann *et al.* 1986; Schupbach & Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987; Mlodzik *et al.* 1987; Winslow *et al.* 1988). The maternal effect of *l(1)ph* in germline clones is included for comparison; the pole hole phenotype shows the maternal requirement for *l(1)ph* while the null phenotype results from lack of both maternal and zygotic *l(1)ph* gene expression. For the genes listed, lack of maternal function produces equivalent posterior alterations of the fate map and identical secondary effects for subsequent posterior CNS and PNS development as described here for *l(1)ph*. Generally for this group, lack of maternal function results in the deletion of A8 through the telson accompanied by the expansion of cell fates in A5 through A7. (In this study, slight variations in the number and/or position of *en*, *ftz* and/or *eve* striped and interstriped regions were also observed in the tails of progeny derived from this group.)

In the anterior terminal region of progeny derived from members of the terminal group, differences in cellular determination are observed. A shift forward of the cephalic furrow and the anterior midgut invagination is shown for embryos derived from all members of this female sterile group except *fs(1)Nasrat*. Consequently, for these embryos instead of the 25–30 cells observed along the longitudinal axis in the heads of wild-type and *fs(1)Nasrat* embryos, only 20–25 cells are present for the progeny derived from *fs(1)pole hole*, *fs(2)torso*, *fs(2)trunk* and *fs(3)torso-like* homozygous mothers. In this regard, the maternal effect of *l(1)ph* is similar to that observed for *fs(1)Nasrat* and differs from the other mutations in this gene class. However, head structures derived from labrum and acron regions are missing or abnormal for embryos produced by all members of the female sterile terminal class group and no additional defects associated with other head segments are observed. When the pattern of *en* staining was compared between different members of this group some embryos derived from homozygous *fs(2)torso* mothers showed additional cells in the gnathal seg-

Table 1. *Comparison between the maternal effect of the terminal female sterile group and the* l(1)ph *mutation*

						l(1)pole hole	
	fs(1)Nasrat	*fs(1)pole hole*	*fs(2)trunk*	*fs(2)torso*	*fs(3)torso*-like	*pole hole*	*null*
Posterior pole hole	+	+	+	+	+	+	+
Posterior extension of the ventral furrow	+	+	+	+	+	+	+
Anterior shift of the cephalic furrow and anterior midgut	−	+	+	+	+	−	−
Posterior deletions A8 through telson (1)	+	+	+	+	+	+	+
Posterior expansion A5 through A7 (2)	+	+	+	+	+	+	+
Twisting of germ band (3)	+	++	++	++	++	+	+
Deletion of labral (4) and acron-derived structures (5)	+	+	+	+	+	+	+
Cell death in head region	++	+	+	+	+	++	+++
Cell death in tail region	++	++	++	++	++	++	+++

In this study, results are based on the analysis of 1 allele for *fs(1)Nasrat*, *fs(1)pole hole* and *fs(3)torso-like* genes; 2 alleles for *fs(2)trunk*, and *fs(2)torso* genes; and 3 alleles for *l(1)ph*. The null phenotype is due to lack of both maternal and zygotic *l(1)ph*$^+$ product. (1) and (2) results based on β-galactosidase expression in embryos containing *ftz/lacZ*, *eve/lacZ* and/or *en/lacZ* constructs (see Materials and methods). (3) Some, but no more than 50%, of the embryos show the twisted germband phenotype (+); more than 50% of the embryos are twisted (++). (4) Labral structures include the labral sense organ or epiphysis and the medial tooth. (5) Acron-derived structures include the brain, and elements of the cephalopharyngeal skeleton.

ments. Presumably, this increase in segmental width is related to the forward shift of the cephalic furrow.

There is also a difference in the percent of progeny that undergo twisting instead of extension of the germ band at 3·5 h of development. For example, greater than 90% of the progeny produced by homozygous *fs(2)torso* females twist at the time of germband extension while less than half of the embryos derived from homozygous *fs(1)Nasrat* females show this defect. Relaxation of these twists occurs prior to dorsal closure in a manner similar to that described for pole hole embryos. Thus, in this respect also the phenotypic affect of *l(1)ph* is equivalent to that of *fs(1)Nasrat*.

Discussion

The *l(1)pole hole* gene plays an important role in the determination and differentiation of cells at many stages of development. Its activity is required in the unfertilized egg and/or in preblastoderm staged embryos for cellular determination in terminal regions of the embryo. When maternal *l(1)ph*$^+$ activity is absent, the labral and acron portions of the head fail to differentiate and, in the tail, deletions include abdominal segments A8–A10 and the telson. The terminal class of female sterile mutations produce equivalent phenotypic changes on embryonic development (Schupbach & Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987). Based on the similarity between the embryonic phenotypes produced by these mutations, the *l(1)ph* gene can be considered a member of the *Drosophila* terminal gene class.

In *Drosophila* embryos, overlapping maternal and zygotic genetic programs are required to ensure the establishment of wild-type segmental identities along the anterior–posterior embryonic axis. For the anterior half of the embryo, a gradient of *bicoid* protein, highest at the anterior embryonic pole, is responsible for specifying cellular identities in gnathal and thoracic segments in a concentration-dependent manner (Driever & Nüsslein-Volhard, 1988*a*). Also, *bicoid*$^+$ activity is required to distinguish between head and tail pathways of differentiation in the terminal region of the head. Embryos that develop without maternal *bicoid* activity show an anterior duplication of the telson, loss of gnathal and thoracic segments, and spreading of terminal and abdominal cell fates into the deleted gnathal and thoracic domain of the embryo (Driever & Nüsslein-Volhard, 1988*b*). For embryos lacking maternal terminal class activity, a normal distribution of *bicoid* protein is observed (Driever & Nüsslein-Volhard, 1988*b*). These embryos show a deletion of terminal head structures accompanied by an anterior shift of the cephalic furrow and anterior midgut invagination (Schupbach & Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987). Some embryos also show an enlargement of the thoracic segments (Winslow *et al.* 1988). However, for embryos lacking maternal *fs(1)Nasrat* or *l(1)ph* activity, spreading of cellular identities toward the anterior pole is not observed. Both the anterior midgut invagination and cephalic furrow form at their wild-type positions in these embryos but labral and acron head structures are missing or are abnormal. This difference in the maternal effect between members of the terminal group indicates that *fs(1)Nasrat* and/or *l(1)ph* gene activities may lie downstream or have a limited function in the terminal class pathway and thereby result in a less extreme embryonic phenotype.

Another aspect of the terminal phenotype, the twisting of the embryo at the time of germband extension, may occur as a direct result of the misplacement of the cephalic furrow during early gastrulation. Both *l(1)ph* and *fs(1)Nasrat* embryos show a decreased tendency to twist when compared to embryos derived from other

terminal group genes. Placement of the cephalic furrow at approximately 70% egg length may be necessary to generate the correct morphogenetic balance required for wild-type movement of the germband in a dorsal and then anterior direction. Twisting of terminal class embryos occurs when the germband begins to extend dorsally, then turns in a ventral direction. As a result of this continued ventral movement of the germband, the entire embryo becomes twisted. When the germband retracts, the twists are relaxed and the wild-type linear form of the embryo is restored.

In the posterior region of all terminal class embryos, cell fates normally found at 0–20% egg length are replaced by the posterior expansion of A5–A7 abdominal identities usually established at 40–20% egg length (Degelmann *et al.* 1986; Mlodzik *et al.* 1987; Winslow *et al.* 1988). The assignment of segmental and compartmental cell identity within the expanded region of terminal group embryos appears to be somewhat random since the width and number of segments established is variable between embryos of the same maternal genotype. These slight variations in segmental patterning may result from establishment of abdominal cellular fates in a region containing a high concentration of posterior morphogen.

At the blastoderm stage, terminal class embryos show 2–3 times the wild-type number of cells contributing to the formation of the terminalmost complete abdominal segment (usually A6). However, at later developmental stages, a wild-type pattern in the PNS is observed within A6. Thus, a process of regulation must occur within the expanded segmental domains. Concurrent with nervous system differentiation, massive cell death is observed in the posterior of terminal class embryos. It seems likely that the mechanism used to generate a wild-type PNS pattern in the enlarged segments is *via* cell death. Perhaps, after all possible cell fates have been assumed, cells left with no identities to fulfill die. Cell death is also observed in the heads of terminal class embryos and is particularly striking in those derived from homozygous *l(1)ph* and *fs(1)Nasrat* eggs. Since there is little or no shift forward of the anterior fate map in these embryos, many more cells are formed in the head. Presumably, due to lack of terminal group activity, these cells cannot assume a viable cell state and die.

In conclusion, the maternal effect of *l(1)ph* is essentially identical to that produced by *fs(1)Nasrat* and similar to other members of the terminal class of female sterile mutations. However, the *l(1)ph* gene is unique when compared to the members of the terminal gene group because its expression is required zygotically as well as maternally.

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