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Region-Specific Defects in *l(1)giant* Embryos of *Drosophila melanogaster*

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Lack of zygotic expression of the *l(1)giant* locus (*l(1)gt;* 3A1), produces embryos with defects in abdominal A5, 6, and 7 and within the head. Scanning electron microscopy at the time of segment formation reveals two regions of defects in the segmentation pattern: anteriorly the labial lobe and thoracic segments T1 and T2 are fused; posteriorly, abdominal segments A5–7 are disrupted. The mature embryo shows incomplete head involution and defects within A5–7; fusion of T1 and T2 is no longer observed. Localized cell death within neural and mesodermal tissues is observed at 7 hr of development; later ventral ganglia, A5–7, are missing. Double-mutant analyses of *l(1)gt* with maternal effect lethal mutations and mutations that generate homeotic, segment number, gap, or segment polarity phenotypes indicate that normal activity of *l(1)gt* is required for differentiation of two embryonic domains: one corresponding to labial, T1 and T2 segments, and the second corresponding to abdominal segments 5, 6, and 7.

INTRODUCTION

The pattern of embryonic segmentation in *Drosophila melanogaster* is under the control of genes which act maternally and/or zygotically. These genes can be classified into four categories. First, maternally active genes establish the spatial body plan along the dorso-ventral and anterior–posterior axes within the egg (Nusslein-Volhard, 1979). This class includes the mutations which lead to the bicaudal (Nusslein-Volhard, 1977, 1979), dorsal (Anderson and Nusslein-Volhard, 1984a), torso (Nusslein-Volhard *et al.*, 1982; Degelmann *et al.*, 1986), and tudor (Boswell and Mahowald, 1985) phenotypes. Second, zygotically active genes affect segment number and polarity (Nusslein-Volhard and Wieschaus, 1980). Included in this group are the segment polarity, pair-rule, and gap mutants. Five loci, *Kruppel*, *hunchback*, *knirps* (Nusslein-Volhard and Wieschaus, 1980), *tailless* (Strecker *et al.*, 1986), and *giant* (Wieschaus *et al.*, 1984a) have previously been described as "gap" mutants. The third category includes the maternally and/or zygotically active genes that specify segment identity (Ouweneel, 1976). Mutations at these loci induce transformation of segment identity and include the homeotic mutation; *Polycomb* (Haynie, 1983), *Antennapedia* (Wakimoto and Kaufman, 1984), and *bithorax* (Lewis, 1978; Sanchez-Herrero *et al.*, 1985). The fourth category includes essential loci with specific maternal effect lethal phenotypes such as *l(1)pole hole*, (Perrimon *et al.*, 1985), *l(1)hopscotch* (Perrimon and Mahowald, 1986b), and *l(1)dishevelled* (Perrimon and Mahowald, 1986c), reviewed in Perrimon and Mahowald (1986a).

Here, we describe the developmental genetics of amorphic mutations at the *X*-linked *l(1)giant* [*l(1)gt*] locus. Embryos lacking $l(1)gt^+$ activity die during embryogenesis and exhibit a gap phenotype, in which the head is not fully involuted and three abdominal denticle belts are missing. We have (1) determined the earliest time in embryonic development at which the absence of $l(1)gt^+$ gene activity produces abnormal development, and (2) examined whether *l(1)gt* interacts with known loci involved in embryonic patterning. Our results suggest that the *Drosophila* embryo is subdivided into blocks or domains. The normal development of these regions is under the control of zygotic genes which act independently of segment identity. *l(1)giant* represents one of the genes which control two such embryonic domains.

MATERIALS AND METHODS

1. Strains. Three embryonic lethal alleles at the *X*-linked *l(1)gt* locus have been examined. The gt^{x11}, gt^{Q292}, and gt^{13z} alleles were maintained on marked chromosomes in females heterozygous for the *l(1)gt* mutation and a balancer chromosome (*FM7/y sc* gt^{x11} w^a, *FM6/y sc* gt^{x11}, *FM6/*gt^{x11}, *FM7/y* gt^{Q292} *rst*). Alternatively, the *l(1)gt* mutation was carried in males of an attached stock [*C(1)DX, y f/y sc* gt^{x11} w^a/*Dp(1; Y)*w^{+303}, or *C(1)DX, y f/* gt^{13z}/w^+Y]. The gt^{Q292} allele was obtained from T. Kaufman, and the gt^{13z} allele was provided by B. Judd. All experiments were performed at 25°C, unless stated otherwise.

The *X*-chromosome deficiencies *Df(1)Pgd kz, Df(1)2F1-3A4, Df(1)X12, Df(1)278.4b.1a, Df(1)62g18* and *Df(1)-TEM75*, have been previously described (Perrimon *et al.*, 1984, 1985). The deficiencies *Df(1)*w^{rj1} and *Df(1)65j26* were provided by B. Judd (Shannon *et al.*, 1972).

We confirmed the map location of *l(1)gt* (Kaufman *et al.*, 1975) to be within polytene chromosome band 3A1

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by several crosses that utilized a series of overlapping deficiencies. The deficiencies *Df(1)w*^{rj1} and *Df(1)65j26*, which have distal breakpoints in 3A2, complemented *l(1)gt*. The deficiencies *Df(1)62g18* and *Df(1)TEM75*, which have distal breakpoints in 3A1-2 and 2F5-3A1, respectively, uncovered *l(1)gt*.

We determined that three embryonic lethal alleles at the *l(1)gt* locus (*gt*^{x11}, *gt*^{Q292}, and *gt*^{13z}) produce an extreme *l(1)gt* phenotype and are amorphic in character, based on the gap phenotype observed in cuticle preparations of unhatched embryos homozygous or hemizygous for each of these alleles.

To test for possible genetic interactions between *l(1)gt* and other loci involved in the establishment of embryonic pattern, we constructed double mutants between the *gt*^{x11} allele and one mutation within the class of the maternal effect lethal (MEL) loci, or the zygotically active gap, pair-rule, segment polarity, or homeotic loci. Double-mutant combinations were constructed between *gt*^{x11} and the MEL mutations *bicaudal* (*Bic*$^{D71.34}$), obtained from J. Mohler and E. Wieschaus, or *fs(1)Nasrat*^{211} [*fs(1)N*^{211}], isolated in our laboratory. Among the embryonic lethal loci, we tested the gap mutants *Kruppel* (*Kr*^{2}), *hunchback* (*hb*^{14f21}), and *knirps* (*kni*^{5F107}), the segment polarity mutations *patch* (*ptc*^{IN108}), and *gooseberry* (*gsb*^{IIX62}), and the pair-rule mutations *odd-skipped* (*odd*^{IIID36}), *even-skipped* (*eve*^{ID19}), *engrailed* (*en*^{IIB86}), and *paired* (*prd*^{IIB42}). The homeotic mutation *Polycomb* (*Pc*^{3}) and deficiencies for the *Polycomb* [*Df(3L)Pc, and* Ultrabithorax [Df(3R)Ubx^{109}] loci were tested. Double-mutant embryos were obtained between *gt*^{x11} and each of these mutations utilizing standard genetic crosses. Stocks were obtained from the Bowling Green Stock center.

Details on the balancers and markers used are described in Lindsley and Grell, (1968).

2. Preparation of embryos. For cuticle examination, embryos were dechorionated in 50% bleach for 3–5 min, rinsed in water, and incubated at 60°C in glycerol/acetic acid (1/4) for at least 2–3 hr. Embryos were then mounted on slides in Hoyer's solution and allowed to clear at 60°C (van der Meer, 1977).

Histological sections were prepared as described in Mahowald *et al.* (1979). Briefly, *FM7/y sc gt*^{x11} *w*^{a} embryos were collected and aged at 25°C for the appropriate length of time. They were dechorionated and rinsed in water as described above, and the vitelline membrane removed according to Dequin *et al.* (1984).

The neural ganglia of *gt*^{x11} embryos 10–13 hr old were stained for acetylcholinesterase activity according to Brown and Schubiger (1981).

For scanning electron microscopic analysis, staged embryos were collected and fixed as described for histological analysis. These were post-fixed overnight at room temperature in 1% OsO_4 and processed for SEM as described (Turner and Mahowald, 1976), except that embryos were mounted on double-stick tape.

RESULTS

1. Early Development of Wild-type Embryos

Because *l(1)gt* mutant embryos exhibit head and thoracic defects during germ band elongation (see below), we reexamined the development of the head region of wild-type embryos in relation to parasegment and segment formation. Martinez-Arias and Lawrence (1985) have postulated that the parasegmental grooves are the earliest morphological evidence of segmentation, and that each parasegment comprises the posterior compartment of one segment and the anterior compartment of the adjacent segment. Later in development, the parasegmental grooves disappear and segmental boundaries are formed at or near the level of tracheal pits (Keilin, 1944; Ingham *et al.*, 1985a; DiNardo *et al.*, 1985).

Scanning electron microscopic (SEM) analysis reveals the presence of grooves along the ventral surface for a short period during germ band extension (Figs. 1A, B; Turner and Mahowald, 1977). We suggest that these grooves, located in between the tracheal pits, correspond to the parasegmental grooves described by Martinez-Arias and Lawrence (1985). During germ band shortening, these grooves are no longer visible and segments are formed (Fig. 1C).

In the cephalic region, the mandibular, maxillary and labial segments can be observed (Fig. 1B). The demarcations between the segments and parasegments in the head (Fig. 1B) are less clearly evident than they are in the thorax and abdomen (Fig. 1A). For example, we note that in the gnathal region, a ventral groove, which we interpret as the groove separating parasegments 2 and 3 (indicated by asterisk in Fig. 1B), is out of register with the boundary between the labial and T1 segments located on the dorso-lateral surface of the embryo. This observation suggests that segment formation occurs very soon after parasegment formation in the head region, and that parasegments form ventrally, while segments initially form more laterally. This second inference is supported by the fact that the tracheal pits, which define segmental borders (Keilin, 1944; Ingham *et al.*, 1985a), are located just posterior to the lateral margins of the grooves that we believe are parasegmental grooves (Fig. 1A). We refer to these grooves throughout the text as the parasegmental grooves.

Ten tracheal pits are observed during germ band extension. The first pit is located within parasegment 4, at the boundary between thoracic segments 1 and 2 (Fig. 1B); the 10th pit is located within parasegment 13 (data not shown). No tracheal pit is associated with the labial and thoracic T1 boundary. The salivary gland invagi-

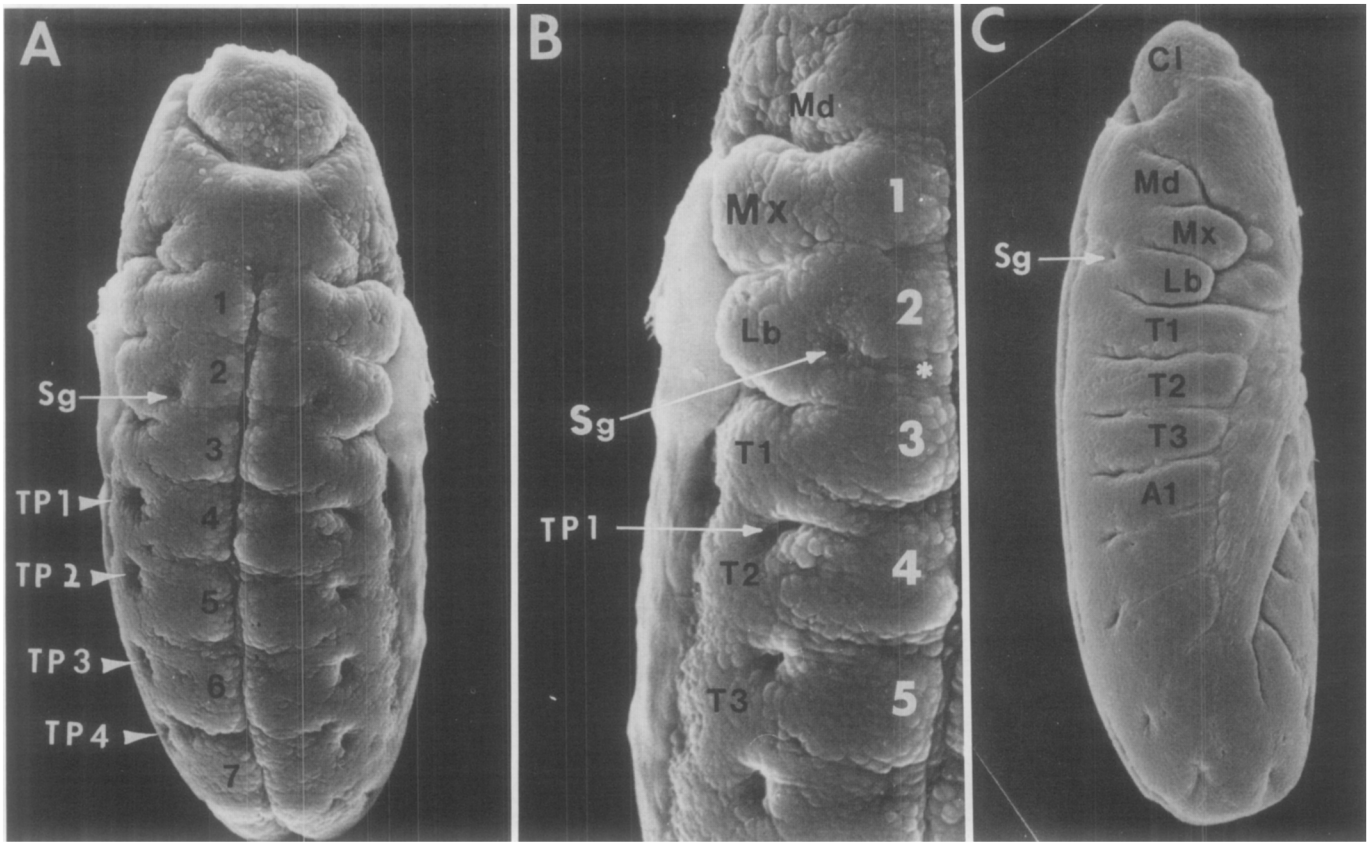


FIG. 1. Scanning electron micrographs of wild-type embryos. (A) An embryo at the extended germ band stage shows parasegmental grooves demarcating parasegments 1-7, and tracheal pits 1-4. 250×. (B) An enlarged detail of (A), which shows that both parasegments 1-3 and the mandibular, maxillary, and labial head segments form in close temporal sequence. Note that the first tracheal pit forms at the thoracic T1 and T2 segmental border. The salivary gland invagination lies within the labial lobe. The asterisk (*) indicates the border between parasegments 2 and 3. 400×. (C) An embryo that has begun germ band shortening has formed the cephalic, thoracic, and first abdominal segment. 250×. Nomenclature: TP, tracheal pit; Sg, salivary gland invagination; Cl, clypeolabrum; Md, mandibular lobe; Lb, labial lobe; T, thoracic segments; A, abdominal segments.

nation is located within the labial lobe, just anterior to the border of parasegments 2 and 3 (Fig. 1B). The spiracle forms independently from the tracheal pits during germ band shortening (Poulson, 1950; Fullilove and Jacobson, 1978; Turner and Mahowald, 1977), and occupies a dorsolateral position within the 8th abdominal segment (Fig. 2B).

2. *Early Development of l(1)gt Embryos*

The early *l(1)gt* phenotype consists of two regions of segmental fusions, correlated with localized cell death. These defects are first evident during germ band elongation and parasegment formation. (Although most SEM analysis utilized the gt^{x11} allele, gt^{13z} and gt^{Q292} mutant embryos had defects similar to those of gt^{x11} embryos. Throughout the text, the amorphic *giant* phenotype will be referred to as *l(1)gt.*) Posteriorly, *l(1)gt* embryos either lack or have displaced tracheal pits 7, 8, and 9 (Fig. 2D). Abnormal folds are present in the cuticular region corresponding to parasegments 10-12 (data not shown). The tenth set of tracheal pits in parasegment 13 are usually present in the mutant. Thus, *l(1)gt* embryos form between 7 and 9 pairs of tracheal pits rather than the normal 10. These structural defects contrast with the well organized parasegmental grooves and tracheal pits observed in normal embryos (Fig. 1A, 2A).

The segmental defects are more striking in *l(1)gt* embryos which have completed germ band shortening. Segmental boundaries do not form correctly within the abdominal A5-A7 region, producing fused or partial segments (Fig. 2E). A spiracle is frequently duplicated in the dorsolateral cuticle of the abdominal A6-A7 region (Fig. 3B). However, the spiracle within the 8th abdominal segment is formed normally.

At the anterior of all mutant embryos, gnathocephalic segmentation is defective. The anterior defects are fully penetrant for the three lethal alleles examined. Thoracic segments T1-2 have failed to separate at this develop-

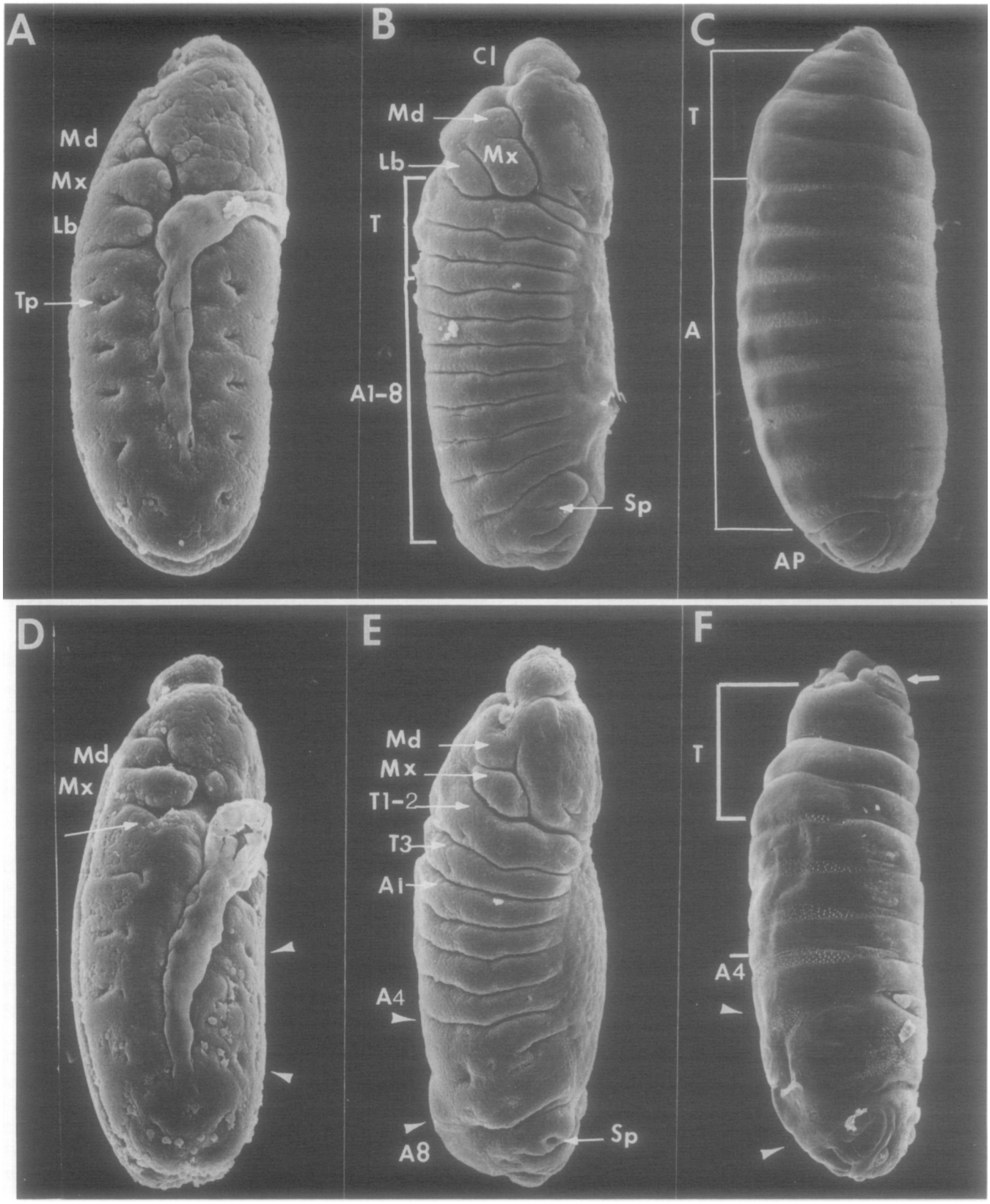


FIG. 2. Scanning electron micrographs of wild-type (A, B, C) and gt^{x11} embryos (D, E, F) at 7, 10, and 12–15 hr, respectively. (D) During germ band extension at 7 hr, the gt^{x11} embryo is apparently missing the labial lobe (arrow) and tracheal pits 8 and 9 (arrowheads). (E) A gt^{x11} embryo that has completed germ band shortening. Segments T1-2 and A5-7 (arrowheads) are fused. The labial lobe is not visible. (F) A gt^{x11} embryo at 12–15 hr of development. The three thoracic segments are present (T), but A5–A7 lack cuticular structures (arrowheads). Head involution is not complete (arrow). All figures at 250× magnification. Nomenclature: Md, mandibular lobe; Mx, maxillary lobe; Lb, labial lobe; T, thoracic segments; A, abdominal segments; sp, spiracle, AP, anal pads.

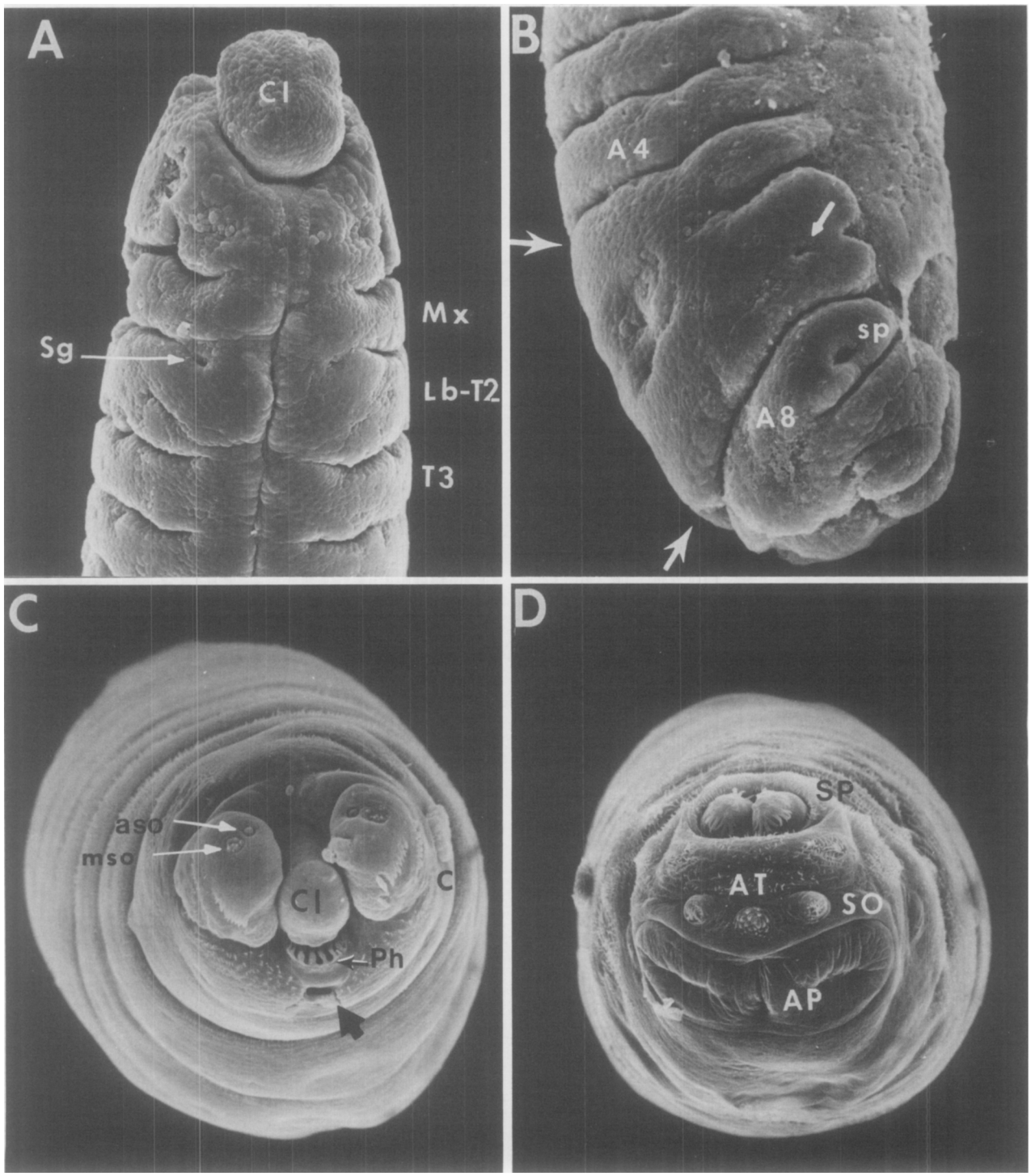


FIG. 3. Scanning electron micrographs showing details of gt^{x11} embryos. (A) A gt^{x11} embryo that has begun germ band shortening has failed to form segmental boundaries between the labial, thoracic T1 and T2 segments. The salivary gland invaginations are present on this defective segment. 250×. (B) An embryo that has completed germ band shortening contains an extra spiracle invagination in the fused region (small arrow). Note the presence of the spiracle in A8 (sp) and the fused A5–A7 region (large arrows). 400×. (C) The anterior region of a 12–15-hr-old gt^{x11} embryo, reveals the salivary gland duct (black arrow) opening on the ventral surface of T1. The clypeolabrum (Cl) and pharyngeal muscles (Ph) have not been internalized. Note the presence of the cirri (C), the antennal, and maxillary sense organs (aso, mso). The first thoracic denticle belt is slightly disrupted. 400×. (D) No defects are detectable in the posterior most structures of a 12–15-hr gt^{x11} embryo. 400×. Nomenclature: Cl, clypeolabrum; Md, mandibular lobe; Mx, maxillary lobe; Lb, labial lobe; T1–T3, thoracic segments T1, T2, T3; sp, spiracle; aso, antennal sense organ; mso, maxillary sense organ; C, cirri; Ph, pharyngeal muscles; AT, anal tuft; SO, sense organs; AP, anal pads.

mental stage (Figs. 2E, 3A). The first tracheal pit is found on this large segment (Fig. 2D). The labial head segment does not separate from the double thoracic segment (Figs. 2E, 3A).

The salivary gland derives from the labial appendages as paired invaginations during normal embryogenesis (Poulson, 1950; Turner and Mahowald, 1979; Fig. 1A, this paper). Since labial segment formation is abnormal in the mutant, we examined the development of the salivary gland in *l(1)gt* embryos. We first detect paired invaginations at 9 or 10 hr of development within or just posterior to the folds formed by the maxillary and defective labial-thoracic segments (Fig. 3A). We interpret these invaginations to be the primordia for the salivary glands. This idea is supported by the fact that the salivary gland invagination can be observed histologically at about 8 hr of development (data not shown). Subsequent development of the salivary glands appears normal; large cells surround a lumen filled with metachromatically stained secretory material in later stages (Fig. 4B). However, the opening of the common salivary gland duct may remain external due to incomplete head involution (Fig. 4B).

Previous experiments have shown that extensive cell death occurs in *l(1)gt* embryos (Honisch and Campos-Ortega, 1982). These results are confirmed by our histological analysis. Regional cell death is observed within the mesodermal and neurogenic tissue of parasegments 10–13, by 7 hr of development. In addition, some cell death is seen more anteriorly in the head and thoracic region. While some cell death occurs in the neurogenic region of normal embryos, it is more extensive in the thoracic and abdominal regions of gt^{x11} as compared to wild-type embryos (data not shown). We do not observe dead cells or gaps in the ectodermal cells that comprise the hypoderm, either with light (Fig. 4B) or transmission electron microscopy (data not shown). Cells containing pycnotic nuclei and vacuolated cytoplasm are found beneath the hypoderm.

3. Late Development of l(1)gt Embryos

The late *l(1)gt* phenotype, when examined in cuticular mounts or by SEM, is characterized by incomplete head involution and a single gap in segmentation pattern. Whereas wild-type embryos 12–15 hr old have completed head involution and have begun to secrete the denticle belts and setae characteristic of each segment (Figs. 2C, 5A), in *l(1)gt* embryos, the ventral abdominal denticle belts and dorsal hairs of A5, 6, 7, and occasionally A8 are missing or disrupted. Bare cuticle is secreted in the region lacking setae (Figs. 2F, 5B). While the morphology of the 8th abdominal denticle belt is variable, the anal pads, anal tuft, sense organs, and spiracles, structures that are derived from segments A8 to A10, are indistinguishable from those of wild-type animals (Fig. 3D). 33–50% of *l(1)gt* embryos (Table 1) have duplicated spiracles on the dorsolateral surface within the gap region (Fig. 5E). On the external surface, this structure consists of hairs surrounding highly refractile material that resembles filzkorper material. In a few embryos, this surface structure is connected internally to a tracheal tube (data not shown). In many *l(1)gt* mutants the tracheal tubes end blindly anterior to segment A5 (Fig. 5D). The extra filzkorper observed in late stage cuticle mounts (Fig. 5E) may correspond to the duplicated spiracle visible in SEM preparations of 10-hr *l(1)gt* embryos (Fig. 3B).

The failure of thoracic segments T1 and T2 to separate at 6–8 hr of development is no longer observed in mutant embryos 12 to 15 hr old. This observation is supported by two lines of evidence. First, SEM preparations show a clearly defined segment boundary between T1 and T2 (Fig. 2F), on both the dorsal and ventral surfaces. These embryos retain the A5–A7 segmental defect. Second, cuticular preparations of unhatched *l(1)gt* embryos have separate thoracic T1, T2, and T3 denticle belts (Fig. 5B), although the first thoracic belt is slightly disrupted (Fig. 3C).

The reason why *l(1)gt* embryos fail to complete head involution is not clear. However, this may result from a failure to separate the labial and thoracic T1 segments (Fig. 2F, 3A). Ventral pits and Keilin's organs, found in the thoracic segments of normal embryos, are usually present in the mutant. SEM analysis confirmed observations made from Hoyer's mounts, that development of the antennal and maxillary sense organs is normal (Fig. 3C). Cirri are usually present, although some mutants contain only one row of cirri rather than the usual two. The cephalopharyngeal skeleton shows some variability in the degree to which it forms, such that in some embryos, the shape is disrupted (Fig. 5C). The H-piece, when present, is disrupted (data not shown). The mouth hooks are always observed, but may be displaced to the lateral part of the head, rather than meeting in the center (Fig. 5C). Incomplete head involution exposes the pharyngeal musculature in some *l(1)gt* embryos, and the clypeolabrum is not completely internalized (Fig. 3C). Histologically, the pharyngeal muscles appear disorganized, perhaps due to defective head involution (data not shown).

The thoracic defects are assumed to be due to *l(1)gt*, and not to other mutations or background effects since the same defects are seen with the original gt^{x11} following extensive recombination, or when the gt^{x11} allele is heterozygous with deficiencies. Moreover, the segmental and structural defects are not allele specific. Two other embryonic lethal alleles, gt^{13z} and gt^{Q292}, when each was ge-

netically combined with gt^{x11} in heterozygous female embryos, were observed to produce defects similar to those of embryos heterozygous for gt^{x11} and the deficiency *Df(1)62g18*.

Histological analysis showed that internally, cell death within the neurogenic region of early gt^{x11} embryos produces a gap in the ventral nerve cord (Honisch and Campos-Ortega, 1982) which can be seen in sections (Fig. 4C) and in acetylcholinesterase-stained 12-hr embryos (data not shown). Two or occasionally three neural ganglia that correspond to A7, 8, and 9 remain posteriorly. In late stage embryos, following condensation of the ventral nerve cord, a portion of the nervous system remains in the posterior region of the embryo (Fig. 4D). Analysis of 3-μm-thick serial sections showed that in some embryos, which contained a gap in the ventral nerve cord, a partial connection was maintained between the anterior and posterior portions of the ventral nervous system. There is no visible defect from anterior cell death on development of anterior portions of the ventral nerve cord, nor do we detect obvious defects in morphology of the brain lobes in gt^{x11}.

The longitudinal muscles associated with abdominal segments A5 to A7 are missing or do not connect properly to adjacent segments (Fig. 4E). The absence of muscles within this region probably results from mesodermal cell death observed in younger embryos, although it may be a secondary defect due to the neural death. The dorsal region of segment A5 normally contains the paired gonad. Although this segment is disrupted in gt^{x11}, the gonads are present. These organs lie directly adjacent to the hypoderm (Fig. 4E), rather than lying internal to the musculature. The presence of the gonad in mutant embryos is in agreement with recent evidence that the gonad is derived from abdominal segment A4 and not A5 (Karch *et al.*, 1985).

Recently, the viable hypomorphic allele of *giant*, initially described by Bridges and Gabritchevsky (1928), was shown to delay the timing and decrease the peak of ecdysteroid hormone activity that occurs at pupation (Schwartz *et al.*, 1984). To determine if any structural defects existed in the ring gland, which synthesizes ecdysone, we compared histologically the structure of this organ in three wild-type (cf. Poulson, 1950) and three gt^{x11} embryos. While the ring gland is clearly evident in 3-μm-thick serial sections of wild-type embryos as a small group of cells at the base of the frontal sac (Fig. 4F), this organ is seen to be reduced in size or disrupted in comparable serial sections of gt^{x11} embryos (Fig. 4G).

4. Amorphic Alleles of the giant Locus

We first compared the cuticular phenotypes of hemizygous gt^{x11}/Y male, homozygous gt^{x11}/gt^{x11} female, and gt^{x11}/Df female embryos heterozygous for gt^{x11} and a deficiency, at 25°C (Table 1), 18°C, or 29°C, to determine if this allele had any wild-type activity. Embryos were scored for the following characteristics: (1) the percentage of embryos with abdominal denticle belts that were either totally or partially missing (scoring A5–A8), (2) the percentage of embryos with extra filzkorper, and (3) the number of embryos with head defects. No significant difference in the cuticle phenotype is observed when *l(1)gt* embryos are collected and aged at 18 or 29°C, as compared to those prepared at 25°C. This result suggests that the *l(1)gt* mutation does not have residual gene activity that can be expressed at the new temperature.

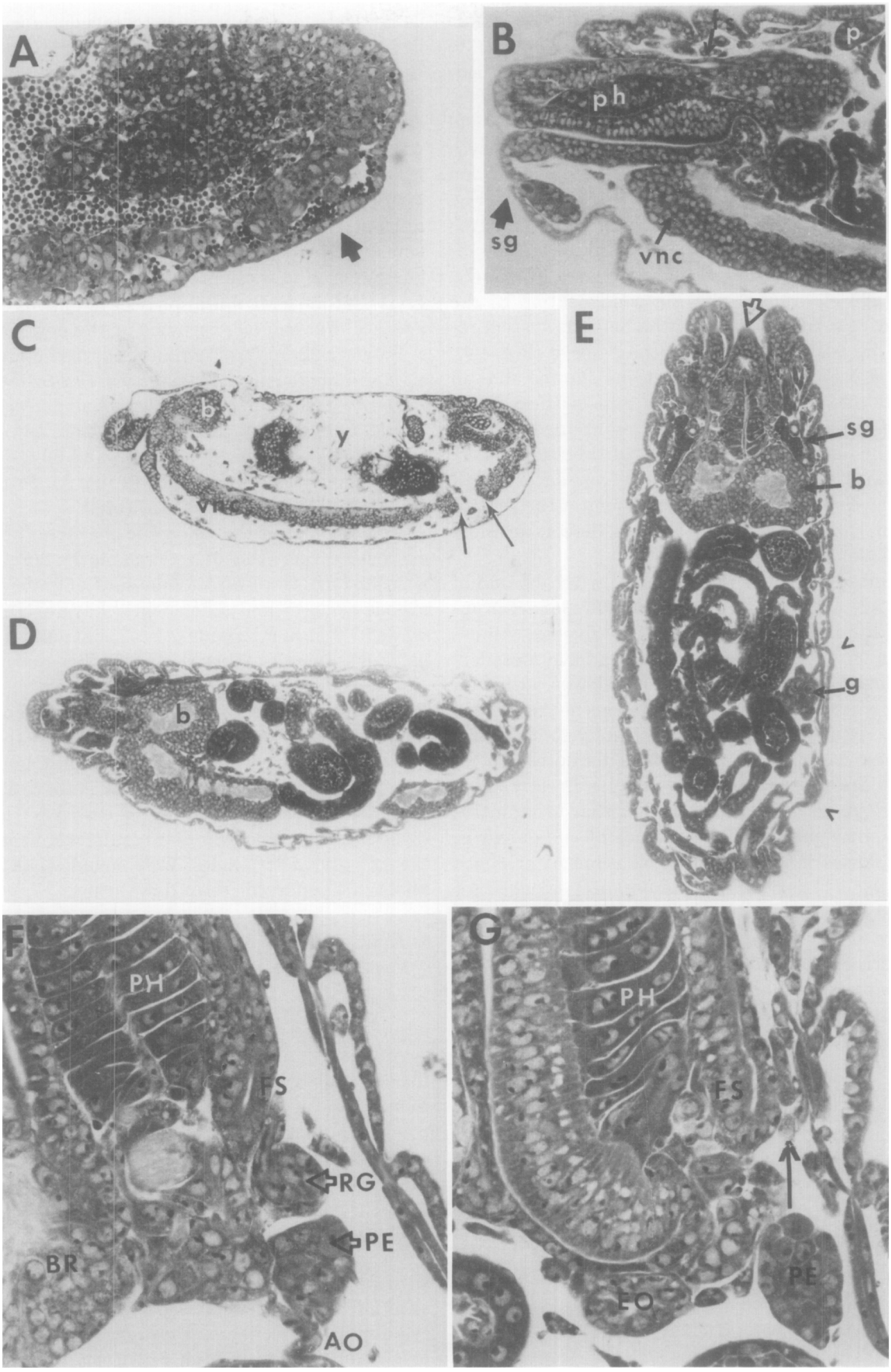
Between 92–100% of female embryos heterozygous for gt^{x11} and a deficiency (*Df(1)X12, Df(1)62g18, Df(1)TEM75, Df(1)2F1-3A4*, and *Df(1)278.4b.1a*) had denticle belts A5, 6, and 7 affected by the gt^{x11} mutation, as compared to 89% of gt^{x11}/Y hemizygous male and 90% of gt^{x11}/gt^{x11} homozygous female embryos (Table 1). In most cases, the denticle belt is severely affected.

Another test of the amorphic character of gt^{x11} is the extra filzkorper (Fig. 5E), found on the dorsolateral cuticle surface. One-third to one-half of embryos, heterozygous for gt^{x11} and a deficiency, have extra filzkorper, while 37% of hemizygous gt^{x11}/Y male and homozygous gt^{x11}/gt^{x11} female embryos contain this structure (Table 1). None of these gt^{x11} embryos complete head involution. Based on these phenotypic criteria, female embryos heterozygous for gt^{x11} and a deficiency do not exhibit a more severe phenotype than do females homozygous or males hemizygous for the gt^{x11} allele, nor is there a significant difference in the percentage of embryos that display these characteristics. Similar results were obtained with the gt^{13z} and gt^{Q292} alleles. We conclude that these three alleles are amorphic or null mutations.

5. Double-Mutant Analysis of l(1)giant: Cell Death Is Region Specific, Not Segment Specific

One hypothesis to explain how *l(1)giant* produces the gap phenotype is that it recognizes and deletes specific segments. An alternative hypothesis is that *l(1)gt* specifies an embryonic domain that is independent of segmental identity and that is deleted when the *l(1)gt* gene product is missing. To distinguish between these possibilities and to determine whether *l(1)gt* interacts with other loci involved in pattern formation, we constructed doubly mutant embryos and examined their cuticular phenotype.

We tested three classes of loci for possible interactions with *l(1)gt:* (1) genes that act maternally to establish the anterior-posterior egg axis (*fs(1)Nasrat*^{211}, *bicaudal*), (2) zygotically active genes that specify segment number (*odd-skipped, even-skipped, engrailed* and *paired*) or an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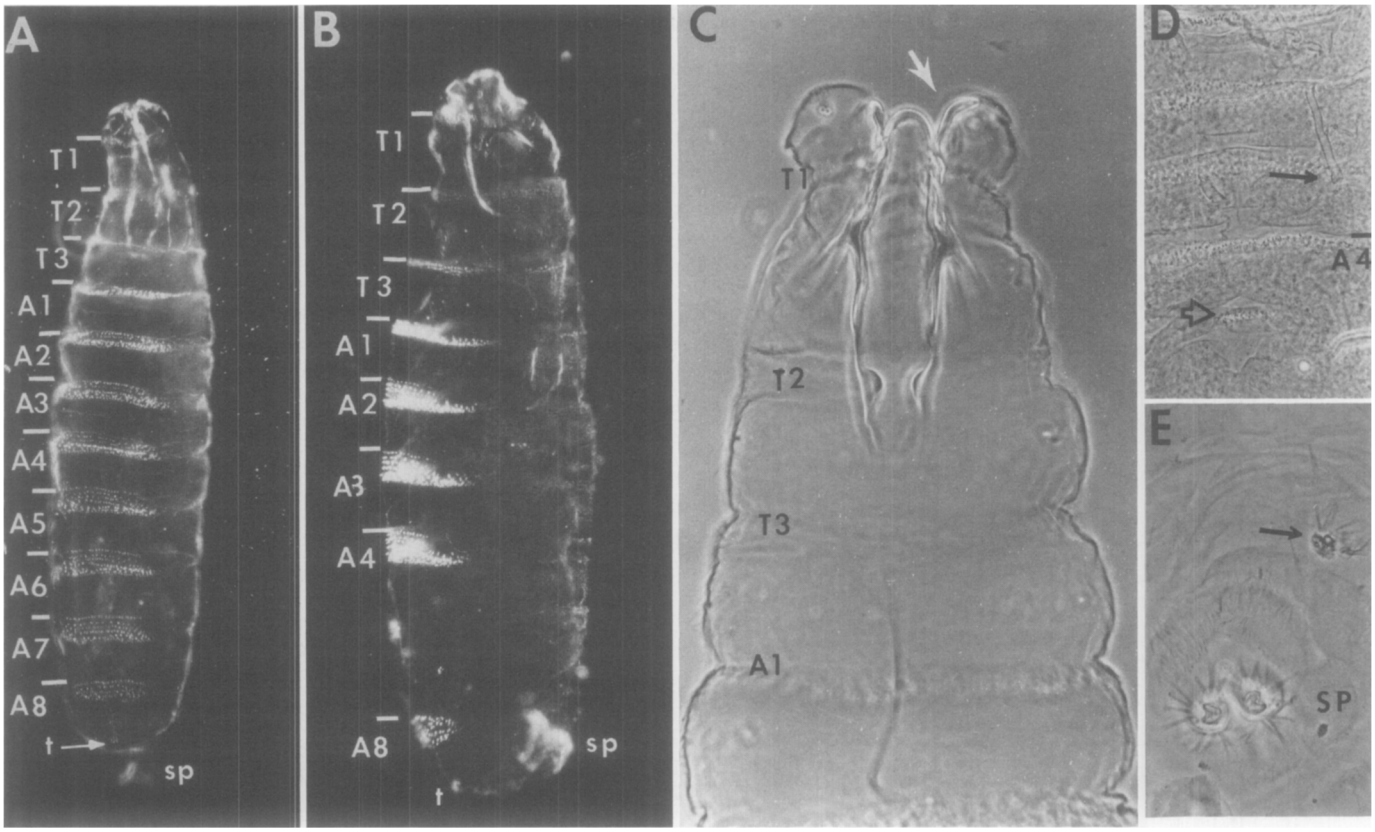

FIG. 5. Cuticular preparations comparing late-stage wild-type and gt^{x11} embryos. (A) Dark field of a wild-type embryo, showing the three thoracic (T1-T3) and eight abdominal segments (A1-A8), the anal tuft (t), and the spiracle (sp). 60×. (B) Dark field of a laterally oriented gt^{x11} embryo is missing abdominal ventral denticle belts in segments A5, 6, and 7. Note the presence of A8 and the spiracles (sp). 60×. (C) Head involution is not completed in the mutant, and the mouth hooks do not assume their normal location (arrow). Note the segmentation of the thorax. 200×. (D) The broken tracheal trunk in a gt^{x11} embryo is shown in relation to segment A4 (small arrow). Note the partial A5 denticle belt (arrowhead). 100×. (E) An extra filzkorper (arrow) is seen on the dorso-lateral surface adjacent to the posterior spiracles (sp) of a gt^{x11} mutant. 200×.

embryonic domain (*Kruppel, hunchback, knirps*), and (3) homeotic loci that are required to define and maintain segmental identity (*Polycomb, Ultrabithorax*). If two mutations independently affect the phenotype, the final cuticular pattern will be a combination of the two individual patterns. If one mutation is epistatic to the second, then only one mutant cuticular pattern will be expressed. However, if a new phenotype occurs, then one function is dependent upon the other or that they are interdependent.

l(1)giant combined with homeotic mutations. Homeotic genes are necessary to establish and maintain the identity of segments. For example, the mutation *Polycomb* (Pc^3) or the deficiency *Df(3L)Pc* produces an embryo with all thoracic and abdominal segments transformed to the A8 cuticular pattern except A9 and A10 (Fig. 6A1).

FIG. 4. Histological sections showing details of gt^{x11} mutants at various developmental stages. (A) A sagittal section of a 9-hr embryo shows cell death beneath the hypoderm in the posterior region of a gt^{x11} embryo (arrow). Note the presence of the large neuroblasts. 100×. Anterior is to the left, ventral side is down in A, B, C, and D. (B) A sagittal section of a 15-hr embryo. The salivary gland duct (sg), opens on the ventral surface of thoracic T1. Note the invagination of the frontal sac (fs), dorsal to the pharynx (ph), the pericardial cells (p) beneath the dorsal hypoderm, and the ventral nerve cord (vnc). 100×. (C) A longitudinal section of a gt^{x11} embryo at 12 hr of development, showing the brain (b) and ventral nerve cord (vnc). A gap is present in the posterior portion of the ventral nerve cord (vnc). 60×. (D) A late-stage gt^{x11} embryo, after the nervous system has condensed, retains a gap in the ventral nerve cord (arrows). 60×. (E) A frontal section of a 17-hr gt^{x11} embryo, showing incomplete head involution (arrow), presence of the salivary gland (sg) and gonad (g), and lack of hypodermal segmentation in the A5-A7 region (arrowheads). Note the disruption of segmental muscles in this same region. 60×. (F) In normal embryos, the ring gland (RG) is a small group of cells attached to the base of the frontal sac (FS). The pericardial cells (Pe), which lie on either side of the aorta (AO), the pharyngeal muscles (Ph), and brain (br) are visible. This section is slightly oblique. 400×. (G) An identifiable ring gland is absent in gt^{x11} embryos (arrow points to region where ring gland should be found). The frontal sac (FS) and pericardial cells (Pe) are correctly positioned. The esophagus (EO) is posterior to the pharynx (Ph). This section is somewhat lateral. 400×.

TABLE I[a]

Genotype	N	% of embryos with n denticle belts affected			% of embryos with extra filzkorper
	n =	4	3	2	
gt^{x11}/Df					
Df(1)2F1-3A4	49	0	92	8	37
Df(1)278.4b.1a	28	0	96	4	50
Df(1)X12	34	0	97	3	41
Df(1)62g18	38	5	95	0	37
Df(1)TEM75	21	0	100	0	43
gt^{x11}/gt^{x11}	135	6	92	2	38
gt^{x11}/Y	201	8	89	3	37

[a] The source and breakpoints for the deficiencies listed have previously been described (Perrimon *et al.*, 1984). *N* represents the total number of embryos analyzed. Segments A5, 6, 7, and 8 were scored for missing or disrupted denticle belts. For $n = 4$, abdominal belts 5, 6, 7, and 8 were affected; $n = 3$ represents belts 5, 6, 7 or 6, 7, 8; $n = 2$ represents belts 5-6, or 6-7. All gt^{x11} embryos had more than one denticle belt affected.

Df(3R)Ubx^{109} deletes a portion of the bithorax complex and results in the transformation of all thoracic and abdominal segments to a T1p/T2a phenotype except T1a. Embryos doubly mutant with either homeotic mutation and gt^{x11} showed missing denticle belts in the region corresponding to A5–A7, but with the remaining segments transformed to A8 (with Pc^3, Fig. 6A2) or T1p/T2a (with *Df(3R)Ubx*^{109}, data not shown). This result establishes that *l(1)giant* affects a posterior region which is independent of segmental identity.

l(1)giant combined with zygotic pattern defect mutations. The result of combining *l(1)giant* with mutations in each of the major pattern classes was examined. Among the segment polarity class, we examined *gooseberry* and *patch*, among the pair-rule mutants we examined *odd-skipped, even-skipped, engrailed*, and *paired*, and among the gap mutants, we analyzed *hunchback, knirps*, and *Kruppel*. In each instance except *Kruppel* the double mutant of gt^{x11} with a pattern-defect mutation produced a cuticular phenotype which is readily interpreted as a combination of the two patterns. In each case, the denticle bands that correspond to A5–A7 were missing (data not shown).

The result with *Kruppel* is especially intriguing. *Kruppel* acts zygotically to produce a major gap in the anterior portion of homozygous embryos so that thoracic and most abdominal segments are missing (Wieschaus *et al.*, 1984b). Concomitant with this gap, a partial mirror image duplication of A6–A8 occurs (Fig. 6B1). The gt^{x11}; *Kruppel* double mutant retains the anterior mirror image duplication except that the cuticular pattern for A6 and possibly A7 are apparently deleted (Fig. 6B2). In conjunction with the double-mutant results obtained with the homeotic mutations, these results suggest that the *giant* gene interprets the *Kruppel* pattern; the posterior abdominal region is recognized, even if it forms anteriorly. A second possibility is that the anterior defect in the gt^{x11}; *Kruppel* double-mutant represents the anterior defect observed in germ band shortened gt^{x11} embryos, and that this defect is not corrected in the double mutant. We feel this is less likely because in late stages, gt^{x11} embryos do form a segmental boundary and denticle belts in thoracic T1 and T2.

l(1)giant combined with maternal effect mutations. The results from double-mutant combinations with the homeotic mutations indicate that *l(1)gt* affects a region of the embryo that is independent of segmental identity. We tested the interaction of *l(1)giant* with two types of maternal effect genes. One mutation, *fs(1)Nasrat*^{211} [*fs(1)N*^{211}], produces a torso-like phenotype that affects the posterior 25% of the blastoderm (Fig. 6C1). Females homozygous for *fs(1)N*^{211} produce embryos that lack posterior endodermal structures and the posterior 8th to 10th abdominal segments. The posteriormost segment formed is a truncated abdominal 7. *In situ* hybridization with a probe to the *fushi tarazu* gene has established that the presumptive posterior abdominal region of the blastoderm is shifted posteriorly (Degelmann *et al.*, 1986). The second mutation, *Bicaudal*, produces a mirror image of the posterior region of the embryo in which the anterior segments are missing and A6–A8 plus analia are duplicated (Mohler and Wieschaus, 1986).

Doubly mutant embryos of the appropriate genotypes were produced and they showed an unexpected phenotype. Although the gt^{x11} mutation normally deletes abdominal segment A7, the doubly mutant *fs(1)N*^{211} gt^{x11} embryo formed a truncated A7 denticle belt, and lacked segments A5, 6, and 8, as well as the spiracles, filzkorper, anal tuft, and anal pads (Fig. 6C2). This pattern was consistently observed in 23 double-mutant embryos. The result suggests that the embryonic axis is shifted posteriorly by the maternal action of *fs(1)N*^{211}, but that the region recognized and deleted by *l(1)gt* is not shifted. This supports the interpretation of results obtained with the homeotic loci, that *l(1)gt* recognizes a specific domain in the posterior embryo which is not dependent on the identity of specific segments.

In the case of gt^{x11} embryos derived from homozygous *Bic*$^{D71.34}$ females, we observed a cuticle pattern similar to that obtained with the gt^{x11}; *Kruppel* double-mutant embryos. Gaps were found in the cuticular pattern, corresponding to A6–A7, in the posterior and anterior regions (Fig. 6D2). In some double mutants, the gap was more extreme at one end than the other, but it was impossible to determine which end was more severely af-

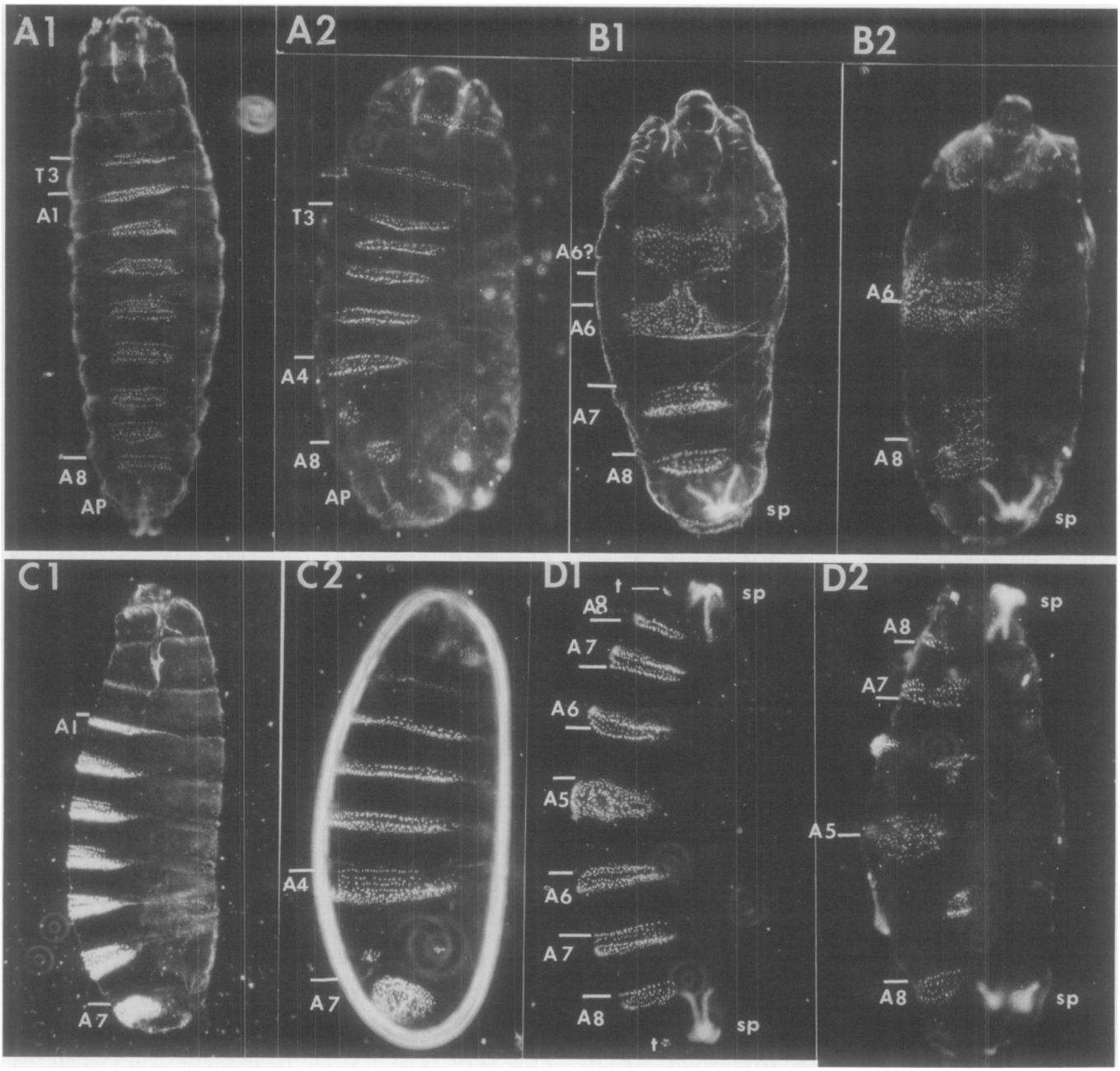

FIG. 6. Cuticular preparations of late-stage double-mutant embryos. (A1) *Polycomb* homozygous embryo. The ventral cuticular pattern shows a transformation toward the A8 pattern. Denticle belts T2, T3, A1, and A2 show a partial transformation, while A3–A7 segments more closely resemble the A8 pattern. T1 is obscured by the mouthparts. (A2) gt^{x11}/Y; Pc^3/Pc^3 embryo. Homeotically transformed segments A5, 6, and 7 are missing. The thoracic denticle belts show a partial transformation toward abdominal characteristics. (B1) *Kruppel* homozygous embryo. Thoracic T1 through abdominal A5 segments are deleted and replaced by a partial mirror image duplication of the 6th abdominal segment. (B2) gt^{x11}/Y; Kr^2/Kr^2 embryo. Two complete denticle belts are present. The posterior A7 denticle belt is partial, while the anterior denticle belt appears to be a fused, mirror image duplication of A6. (C1) *Torso*-like embryo derived from $fs(1)N^{211}$ homozygous females. Abdominal segment A8, the spiracles, anal tuft, and anal pads are absent. (C2) $fs(1)N^{211}$ gt^{x11}/Y embryo derived from $fs(1)N^{211}$ $gt^{x11}/fs(1)N^{211}$ + females. Abdominal segments A5, 6, and 8, the spiracles, anal tuft, and anal pads are missing. (D1) *Bicaudal* embryo derived from homozygous $Bic^{D71.34}$ females. The head, thoracic and abdominal segments A1–A4 are deleted and replaced by a mirror image duplication of abdominal A6, 7, and 8, the anal tuft (t), and the spiracles (sp). (D2) gt^{x11}/Y; $Bic^{D71.34}/+$ embryo derived from $+/gt^{x11}$; $Bic^{D71.34}/Bic^{D71.34}$ females. Two segmental gaps are superimposed on the Bic^{D} pattern. Segments corresponding to A6 and A6-7 are partial or absent. All figures at 60× magnification.

fected. We do not believe the double gap is due to the anterior defect produced by *l(1)gt*, because a segmental boundary forms between thoracic segments T1 and T2 in late-stage embryos.

The double-gap result alone would suggest that *l(1)gt* can recognize specific segments in the posterior abdomen that are duplicated by $Bic^{D71.34}$. However, when taken together with those results obtained from *Polycomb* and $fs(1)N^{211}$, the double gap observed is consistent with the idea that *l(1)gt* produces a region-specific deletion of segments when combined with $Bic^{D71.34}$. Thus, $Bic^{D71.34}$ may regulate the embryonic expression of gt^{x11}.

DISCUSSION

We have characterized the embryonic development of amorphic alleles of the *l(1)giant* (3A1) locus. Histological and scanning electron microscopic analysis has established that an embryonic requirement for expression of the $l(1)gt^{+}$ gene exists in two regions. Two regions with segmental defects are observed at germ band elongation. In the anterior, segmental boundaries do not form between the labial and thoracic T1 and T2 segments, resulting in disruption of head involution and formation of the ring gland. In the abdomen, extensive folds form instead of parasegmental boundaries within parasegments 10-12; two or three pairs of tracheal pits are missing or disrupted and the filzkorper is duplicated. In addition, there is extensive cell death in the ventral nervous system and germ band in this region, resulting in a gap in the ventral nerve cord and the absence of segmental muscles. The usual setae bands and other cuticular differentiations are missing in abdominal 5 through 7.

The salivary gland is derived from the labial lobe (Turner and Mahowald, 1979; Hartenstein *et al.*, 1985). Despite the absence of an identifiable labial lobe in *l(1)gt*, salivary gland invaginations are detectable histologically at about 8 hr of development. At this time they are hidden within a fold between the maxillary and labial-T1 segments, and are not visible with the SEM. By 10 hr of development, however, they are visible on the labial-T1 segment. The fused salivary gland ducts open on the ventral surface cuticle of T1, rather than fusing internally at the ventral midline. Based on the structure of the labial and T1 region in *l(1)gt*, we conclude that the labial lobe is not absent, but is fused with thoracic T1. The salivary glands are probably displaced in 10-hr *l(1)gt* embryos due to this labial-thoracic T1 segmental fusion.

A pair-rule mutation that produces fusions of gnathal segments also disrupts the position of the salivary gland invaginations. In embryos hemizygous for *fushi-tarazu* [*ftz/Df(3R)Scr*] (Wakimoto and Kaufman, 1984), the salivary gland pit is found at a ventromedial position on the fused maxillary-labial segment. Interestingly, the most anterior region deleted by the *ftz* mutation includes the posterior maxillary and anterior labial compartments, corresponding to the second parasegmental unit. Recent data suggested that the regions deleted by *ftz* are parasegmental in origin (Carroll and Scott, 1985; Hiromi *et al.*, 1985). It might be expected that the salivary gland pits would then be deleted by this mutation. However, Ingham *et al.* (1985b) has recently proposed that the boundary of the region deleted by *ftz* is anterior to the anterior/posterior compartment boundary. By this interpretation, the portion of the labial lobe that is removed would fall anterior to the salivary gland invagination. Our SEM analysis of wild type embryos, which shows the salivary gland pits just anterior to the border of parasegments 2 and 3 (Fig. 1B), supports this latter view.

Three *giant* alleles are hypomorphic. Individuals homozygous for gt^{1}, gt^{2} or gt^{E6} (Lindsley and Grell, 1968) produce viable "giant" larvae and adult flies. Recent experiments have shown that third instar larvae homozygous for the gt^{1} mutation are deficient in ecdysteroid activity (Schwartz *et al.*, 1984). Third instar larvae, hemizygous for the gt^{2} or gt^{E6} allele and an embryonic lethal allele (gt^{13z} or gt^{Q292}), have a reduced rate of DNA synthesis in the brain ganglia (Narachi and Boyd, 1985). The relationship between these phenotypes and the regional defects produced by the embryonic lethal alleles (gt^{x11}, gt^{13z}, gt^{Q292}) is not clear. The reduction of the embryonic ring gland in the gt^{x11} embryo suggests a decrease in ecdysone synthesis. In hypomorphic animals, it is possible that the ring gland is abnormal, resulting in decreased ecdysone synthesis and "giant" larvae. The defect in DNA metabolism may be secondary to that of ecdysone. Further experiments will be necessary to clarify the relationships between these defects.

The relationship between cell death and the lack of cuticular differentiation in segments A5 to A7 is not clear. Excessive death of both neural and mesodermal cells occurs in this region at the same time that the abnormal folds within parasegments 10-12 are evident. Subsequently, following germ band shortening and condensation of the nervous system, a gap is visible in the ventral ganglia and no differentiated segmental muscles appear in A5 to A7. Interestingly, fusion of some ventral ganglia occurs in some embryos, but condensation is not completed. Cuticle forms in the A5-A7 region but lacks typical setae and sense organs. It is known from the analysis of weak alleles of *gastrulation defective* embryos (Konrad and Mahowald, in preparation) that cuticular differentiation does not require mesodermal components. Thus, it is possible that the absence of neurons in A5 to A7 is responsible for the lack of cuticular dif-

ferentiation. Our results indicate that *l(1)gt*$^+$ gene activity is required in the neural cells that form the ventral nerve cord and in the mesodermal cells which form the segmental muscles, because early cell death is observed in these two tissues.

A summary of defects observed in *l(1)giant* embryos is given in Fig. 7B. Briefly, defects in parasegments 3, 4, and 10–13 of early embryos result in the removal of segmental boundaries, producing segment fusions in later stages. However, the effects on parasegments 4 and 13 are less severe. Anteriorly, the first thoracic denticle belt is only slightly disrupted and the fusion in T1–T2 is corrected in late stage embryos. A segmental boundary often forms between A7 and A8, and the spiracle forms correctly. In about 5% of mutant embryos, the 8th belt is affected, supporting the idea that the mutation has a weaker effect on parasegment 13. Interestingly, filzkorper and spiracles are duplicated on the dorsolateral surface within the gap region in at least one-third of all *l(1)gt* embryos.

That *l(1)giant* acts within a regionally specific domain which is independent of segment identity was demonstrated by two sets of double mutant experiments. First, the change in segmental identity by homeotic mutations did not provide information that would rescue abdominal segments 5, 6, and 7 deleted by *l(1)gt*. Second, maternal effect lethal (MEL) mutations were used to shift the anterior–posterior axis in the oocyte. The results from embryos doubly mutant for *giant* and a torso-like mutation most clearly demonstrated that *l(1)gt* recognizes a domain in the posterior portion of the embryo. Although the posterior region of the egg was shifted posteriorly by the maternal action of *fs(1)N*^{211}, the regional domain along the length of the embryo that is recognized and deleted by *l(1)gt* was not shifted.

The double-mutant embryos constructed from *l(1)giant* and the *bicaudal* or *Kruppel* genes gave similar phenotypes. The embryonic pattern defect produced by *Kruppel* resembles that of *bicaudal*, but the *Kruppel* gene has no maternal effect (Wieschaus *et al.*, 1984b). We interpret

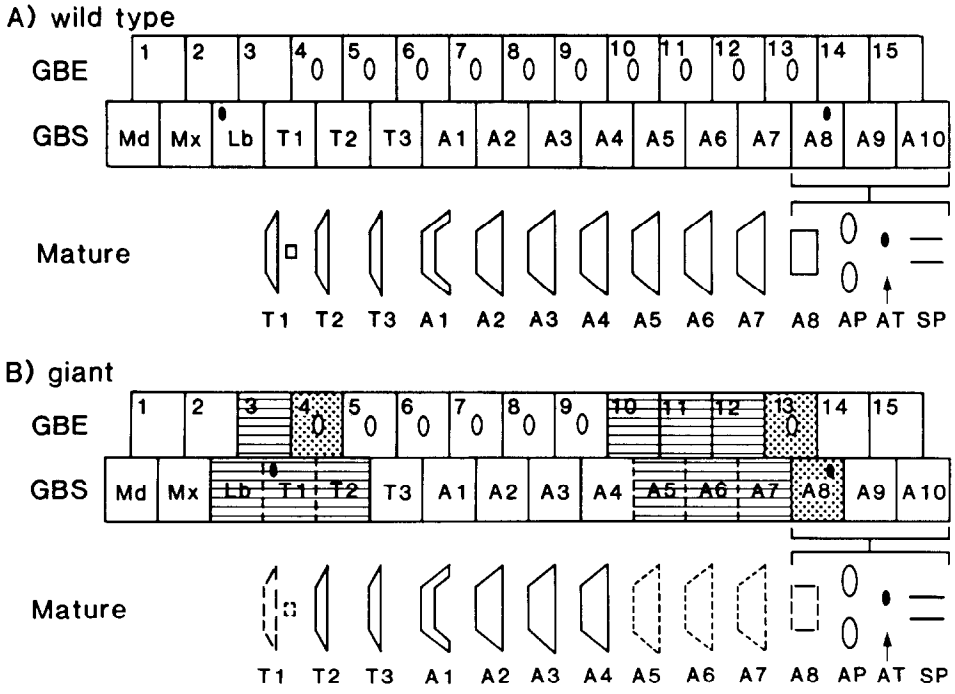

FIG. 7. Diagram comparing the development of parasegments and segments in wild-type and *l(1)giant* embryos. (A) Wild-type embryos contain 15 parasegments (boxes 1–15) extending from the posterior mandibular (Md) through anterior abdominal A10 segments at the stage of germ band elongation (GBE). Parasegments 4–13 each contain a tracheal pit (open ovals). Segmental boundaries form at or near the level of the tracheal pits during germ band shortening (GBS), to form segments (boxes Md through A10). Darkened circles represent the salivary gland invagination in the anterior portion of the labial lobe, and the spiracle in A8. The mature embryo contains 3 thoracic and 8 abdominal denticle belts (trapezoids) at the anterior margin of each segment. (B) The *l(1)giant* mutation has a strong effect on parasegments 3, 10–12 (striped boxes) and a weaker effect on parasegments 4 and 13 (stippled boxes) in GBE embryos. Tracheal pits in parasegments 10–12 are absent or disrupted. During GBS, fused regions are found anteriorly over the labial, and thoracic segments T1 and T2, and posteriorly over the abdominal segments A5, 6, 7, and occasionally A8 (striped boxes). In mature embryos, thoracic T1 is disrupted, and abdominal A5–A8 are missing or disrupted (trapezoids with dotted lines). The effect on denticle belts T1 and A8 are somewhat less severe (trapezoids with broken lines) than on that of A5–A7. Brackets over segments A8–A10 in both wild-type and mutant embryos designate the analia form from these segments, but that these structures are reorganized during complex cell movements (Turner and Mahowald, 1979). Nomenclature: AP, anal pads; AT, anal tuft; SP, spiracles.

the double-gap embryos obtained in each case to indicate that both genes duplicate the posterior domain in which *l(1)gt* is active. *l(1)giant* then recognizes both regions and deletes the A6–A7 denticle belts. These results indicate that *bicaudal* and *Kruppel* regulate the pattern of expression of *l(1)giant* in the embryo. It is unlikely that the anterior defect in these double-mutant constructions, with *Kruppel* or *bicaudal*, is due to the thoracic defect in *l(1)gt*, because late-stage *l(1)gt* embryos always contain thoracic T1 and T2 denticle belts. The result is consistent with the idea that *l(1)gt* removes the ability to form normal segments in the A5–A7 region in a manner that is independent of segment identity.

Of the four gap mutants, only *Kruppel* has been well characterized. The comparison of *l(1)giant* with *Kruppel* suggests that these two gap loci are similar in their temporal action and region specificity. Genetic experiments demonstrated that neither *l(1)gt* nor *Kruppel* are maternally expressed (Perrimon *et al.*, 1984; Wieschaus *et al.*, 1984b). Molecular analysis of *Kruppel* transcript distribution in syncytial blastoderm embryos demonstrated (1) that the *Kruppel* gene is active prior to the completion of the pre-blastoderm divisions, and (2) that *Kruppel* is expressed in specific regions of developing embryos until the stage of germ band extension (Knipple *et al.*, 1985). The region specific pattern of *l(1)giant* is also set up prior to blastoderm formation. Preblastoderm *l(1)gt* embryos, which have been immunostained with an antibody against the *fushi-tarazu* (*ftz*) gene product, show a disrupted stripe pattern (S. Carroll and M. Scott, personal communication), indicating that the $l(1)gt^{+}$ gene is active very early in development.

The embryonically active gap loci, *giant*, *Kruppel*, *knirps*, *hunchback*, and *tailless*, each affect distinctive and sometimes overlapping regions of the embryo. It is possible that the combinatory action of the gap loci generates a molecular environment that triggers or allows the differential expression of other classes of zygotically acting genes (such as the pair-rule loci), to produce the segmentation pattern in the mature embryo.

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