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The development of *fused*⁻ embryos of *Drosophila melanogaster*

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SUMMARY

The mutant *fused* (1–59·5) belongs to a class of lethal mutations in *Drosophila melanogaster* that produce pattern duplications in every segment of the mature embryo. A study of the embryonic development of *fused*⁻ embryos derived from *fused*⁻ mothers shows that extensive cell death occurs early in development. This cell death accounts for the smaller size of the segments in *fused*⁻ embryos. The pattern duplication observed is, probably, a secondary consequence of the pattern deletion.

INTRODUCTION

Segments are the units of pattern for all insects. Their number and identity are invariant characteristics of every species and, for this reason, description of the emergence and diversification of segments is central to the understanding of embryonic development in these animals (reviewed in Sander, 1976; Lawrence, 1981). In *Drosophila melanogaster*, at around the blastoderm stage, the embryo is divided into equal-sized groups of cells (polyclones) which will develop somewhat autonomously and give rise to compartments; segments are pairs of compartments (Garcia-Bellido, Ripoll & Morata, 1973; Wieschaus & Gehring, 1976; Lawrence, Green & Johnston, 1978; reviewed in Lawrence, 1981). At the same time that these polyclones are determined, they acquire certain combinations of active genes which determine the pattern of each segment (Lewis, 1978; Struhl, 1982, 1983; reviewed in Lawrence & Morata, 1983).

Segments are homologous units of lineage (Lawrence, 1981) whose final pattern is expressed upon an underlying morphological theme common to all segments. For example, in the larva of *Drosophila*, the posterior part of each segment is naked and the most anterior part contains several rows of denticles. The precise shape of these denticle belts is characteristic of every segment, but the location and orientation of the denticles are the same from one segment to another. Between the boundaries of a segment a morphogenetic gradient may exist whose scalar values determine the positioning of the pattern elements (Lawrence, 1973) i.e. the gradient of positional information (Wolpert, 1969). The slope of the gradient imposes an important property upon the pattern: polarity. Cuticular structures such as hairs, bristles,

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trichomes and sensory organs, often show an orientation that is thought to be determined by the gradient (Lawrence, 1973).

Very little is known about the biochemical or genetic bases of positional information. In *Drosophila*, mutants have been identified with phenotypic alterations consistent with its existence. These alterations involve the main body axes of the embryo–coordinate mutants (Nüsslein-Volhard, Wieschaus & Jürgens, 1982.) – or the axes of the basic repeat unit, the segment – segment polarity mutants (Nüsslein-Volhard & Wieschaus, 1980; Nüsslein-Volhard *et al.* 1982). In mutants of the latter class, the segments are smaller and display a duplication of the wild-type anterior pattern in the part of the segment which persists.

The mutant *fused* (1–59·5) is an example from this group. Fused$^-$ zygotes develop the lethal phenotype only if their mothers are homozygous *fused*$^-$ i.e. it is a maternal effect mutation, zygotically rescuable. It is known that the fused$^+$ product is required during embryogenesis (Counce, 1956; Fausto-Sterling, 1971; Holmgren, pers. comm.). The present study attempts to correlate the embryonic development of *fused*$^-$ embryos with the segment polarity phenotype. A substantial part of all thoracic and abdominal segments is deleted in *fused*$^-$ larvae. Here, I report cell death in early stages of the development of *fused*$^-$ zygotes and correlate it with pattern deletions as well as with the observed pattern duplications.

MATERIALS AND METHODS

(a) Flies and embryos

Different *fused* alleles were used in these studies, and all of them were kindly provided by R. Holmgren. All the histological analyses were performed with two alleles: fu^1 and fu^{10C}; fu^1 has been described before (Nüsslein-Volhard & Wieschaus, 1980) and fu^{10C} is a weak allele isolated by R. Holmgren. The cuticular analyses were performed with the above mentioned alleles and with fu^{94} and fu^{1A4}. The first one is a strong allele and fu^{1A4} is a deficiency for the locus. Both alleles were isolated by R. Holmgren. To obtain *fused*$^-$ embryos, virgin females of the genotype $y\,w\,ffu^x/y\,w\,ffu^x$ or $y\,w\,ffu^x/y\,w\,ffu^y$ (where x and y are different alleles) were selected from appropriate balanced stocks, and mated to $y\,w\,ffu^{x\text{ or }y}$ males. All the embryos from such crosses are homozygous for *fused*. The wild-type embryos used in this study were from a Canton S stock. Chromosomal markers are described in Lindsley & Grell (1968).

For cuticular preparations, eggs from the appropriate parents were collected on agar plates over 12 h periods and then allowed to mature for 24 h. To obtain embryos of different ages, eggs were collected for 3 h and then allowed to develop to desired times.

(b) Cuticle preparations

24 h-old embryos were dissected out of the vitelline envelope with tungsten needles and then fixed and mounted according to the method of Van der Meer (1977). The cuticle of the wild-type first instar larva has been thoroughly described (Lohs-Schardin, Cremer & Nüsslein-Volhard, 1979; Struhl, 1983); these descriptions have been used as a reference.

(c) Histology

Araldite sections

Embryos of the desired age were dechorionated and fixed according to the method of Zalokar & Erk (1977): dechorionated embryos were placed in heptane saturated with a 0·1M-Hepes buffer pH 7·5; 25% glutaraldehyde; 10% formaldehyde solution. After 10 mins the vitelline

envelope was removed with tungsten needles and the embryos were placed in a 5 % solution of glutaraldehyde in 0·1M-Hepes buffer, pH 7·5, for 2 h at room temperature or overnight at 4 °C. Embryos were then washed with buffer and postfixed in 1 % osmium tetroxide in 0·1M-Hepes buffer at 4 °C. After 2 h, they were thoroughly washed with buffer, dehydrated through a graded series of alcohols embedded in Araldite and processed for sectioning. Sections 2–4 μm thick, were cut on a Porter-Blum microtome and stained with 1 % toluidine blue in 1 % borax.

Fuchsin wholemounts

The method of Zalokar & Erk (1976) as modified by Jimenez & Campos Ortega (pers comm) was utilized. After dechorionation, embryos were prefixed for 10 mins in heptane saturated with 95 % ethanol and 5 % acetic acid. Their vitelline envelopes were removed and the embryos were quickly placed in 95 % ethanol 5 % acetic acid for 2 h at room temperature. They were then rinsed thoroughly with 70 % ethanol and water and placed in 2M-HCl at 65 °C for 10 or 15 mins; washed with 5 % acetic acid and placed in a 2 % solution of fuchsin in 5 % acetic acid. After fifteen minutes, the embryos were cleared in 5 % acetic acid, dehydrated and mounted in Araldite.

The study was performed with 50 embryos of different ages sectioned in chosen orientations and a large number of embryos stained with fuchsin. The preparations were observed under bright-field or Nomarski optics, as required. The embryonic development of *Drosophila* has been described by Poulson (1950) and reviewed by Campos Ortega (unpublished). These works were used as a reference throughout the present study.

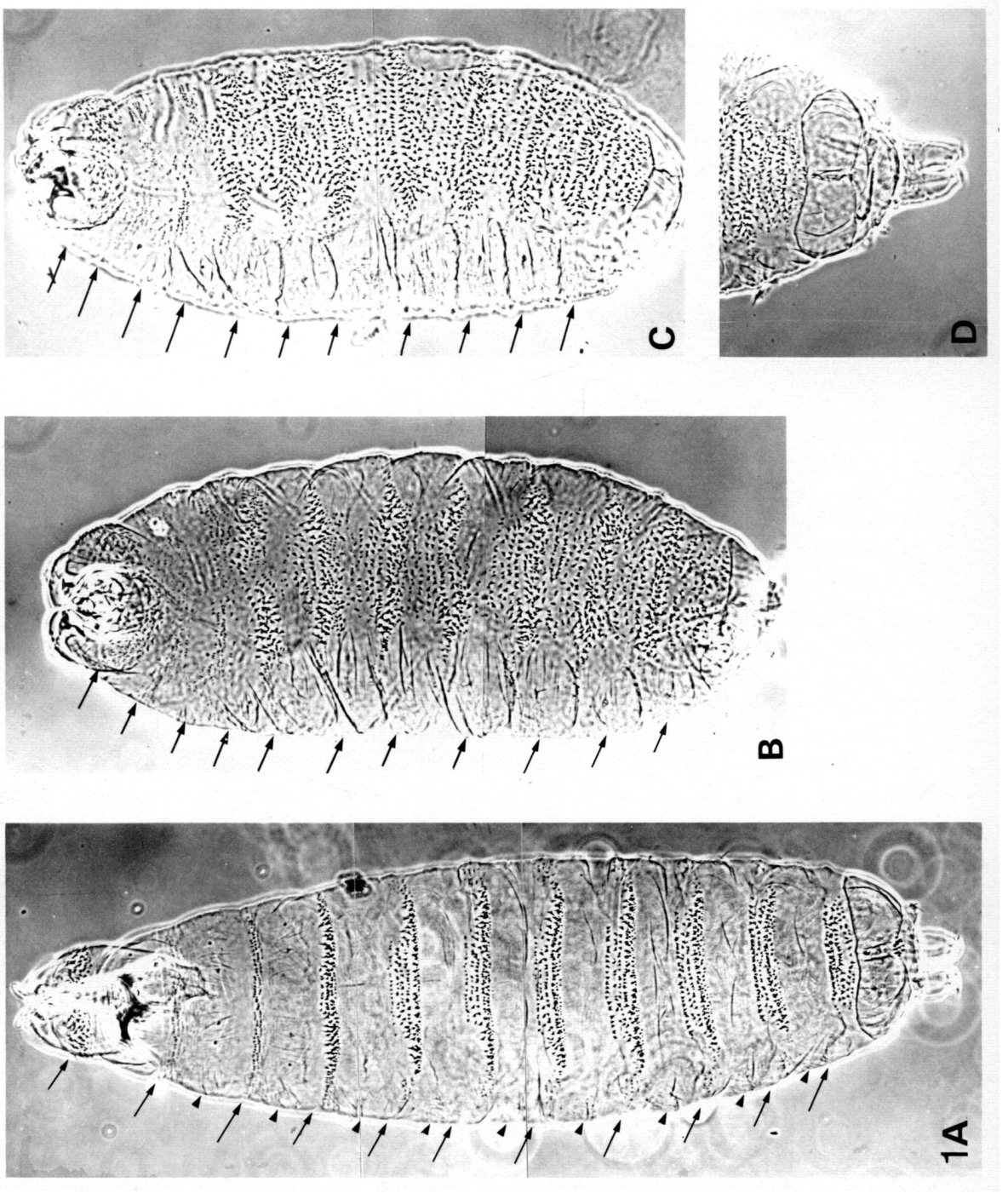
RESULTS

a) The phenotype of fused$^-$ *embryos*

In the wild-type larva, ventrally, part of the anterior compartment of every segment is covered with rows of denticles of rather precise number and orientation (Figs 1,2); the posterior compartment is naked. Dorsally, the cuticle is covered with trichomes and bristles whose shape and spacing permit a distinction of abdominal and thoracic segments. In the abdominal segments, segment boundaries lie in the neighbourhood of the first rows of denticles; in the thorax it is probable that their position is more anterior to this row. The Keilin's organs (Keilin, 1915) provide a landmark for the anteroposterior (A/P) compartment boundary in the thorax (Struhl, 1984). A similar reference for the abdominal segments has not been found.

The phenotype of *fused*$^-$ embryos depends on the genotype of their mothers. The segment polarity phenotype is only expressed by *fused*$^-$ zygotes if their mothers are homozygous mutants. *Fused*$^-$ embryos from heterozygous females develop into adults that show, as their most obvious phenotype, an abnormal wing vein pattern: veins III and IV are fused, to a greater or lesser extent, from the crossvein (absent in general) to the distal tip of the wing. Both *fused*$^-$ males and females are subfertile. In the case of the females, it is known that this reduced fertility is due to the presence of abnormal ovaries (King, Burnett & Staley, 1957) and varies from one allele to another (Wurst & Hanratty, 1979 and own observations).

The phenotype of *fused*$^-$ embryos from *fused*$^-$ mothers with hypomorphic alleles is similar in all thoracic and abdominal segments (Fig. 1). The segments are smaller; ventrally, the wild-type denticle belts are often present but, behind them, additional rows of denticles appear which have a reversed polarity (Figs 1, 2). The first row of extra denticles always is adjacent to the last row of denticles with wild-type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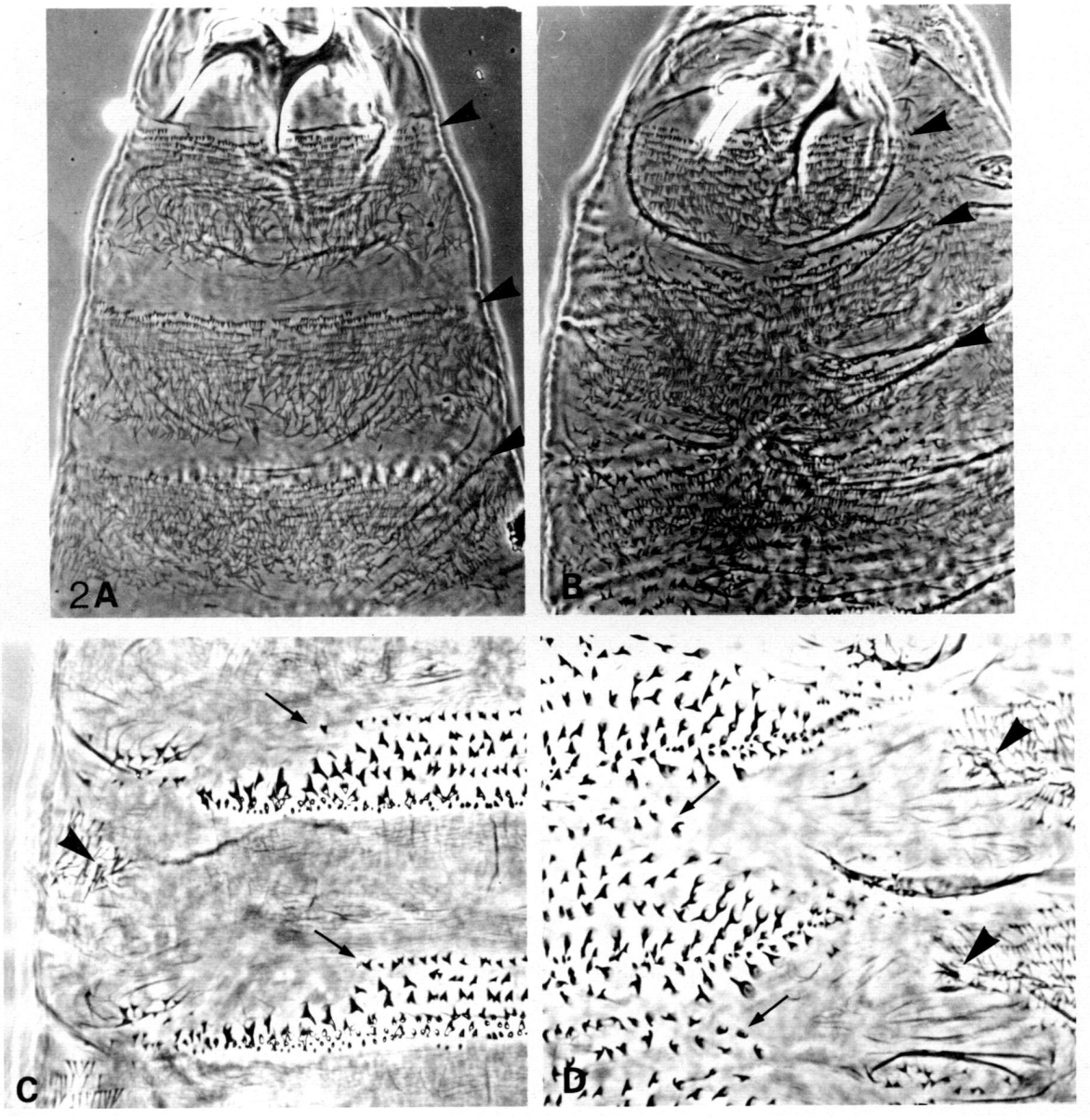
polarity. The number of denticles with reversed polarity seems to be related to the size of the mutant segment: in larvae bearing weak alleles it is possible to observe segments with a wild-type size which do not show any extra denticles. In these same animals, segments with a small deletion show a few extra denticles (Fig. 3A); when the deletion is very large, the remaining posterior part of the segment is covered with denticles and pattern distortions can be also observed in the anterior region (Fig. 3B). A characteristic of *fused*$^{-}$ embryos is their lack of Keilin's organs and



For legends to Figs 1 and 2 see p.104

ventral pits in the thoracic segments. Dorsally, the pattern is variable: *fused*$^-$ larvae often display a wild-type pattern but, at times, they show a deletion of the posterior part of every segment as well as defects along the dorsal midline; occasionally it is possible to observe a few polarity reversals (Fig. 2).

The head of *fused*$^-$ embryos is abnormal due to pattern deletions in the gnathal and cephalic segments homologous to those that take place in thoracic and

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Figure 1. Ventral aspect of cuticular preparations of 24 h-old embryos.

(A) Wild type (for details of the main morphological details see Lohs-Schardin et al., 1979 & Struhl, 1983). Arrows indicate segment boundaries. Arrowheads indicate the approximate positions of the A/P boundary in every segment; this position relies, on the thorax, in the position of the Keilin's organs (Struhl, 1984) and in the abdomen, on the lateral sense organ (Fig. 2).

(B) *fused*$^-$ embryo from a cross : $y\,wffu^{10C}/y\,wffu^{1} \times y\,wfhu^{1}$. It represents the characteristic weak phenotype. Arrows as in (A). In some segments, the most anterior ones, there is a space of naked cuticle between the last row of denticles with reversed polarity and the first row of denticles of the next segment – see the first abdominal segment. Notice that the majority of denticles with reversed polarity lie immediately behind the wild-type rows and that, sometimes, the first row of any segment is rather distorted.

(C) *fused*^{1} embryo from a cross : $fu^{1A4}/y\,wffu^{1} \times y\,wffu^{1}$. The whole surface of the animal is covered with denticles. The anterior rows of wild-type denticles are very distorted but their existence is still clear in some segments. In these animals the posterior compartment is virtually non existant.

(D) Posterior region of a *fused*^{1} embryo similar to that shown in (B). Anal pads and posterior spiracles are well formed. Magnifications: all × 128.

Figure 2. Dorsal and ventral details of first instar larvae.

(A) Dorsal view of the cuticle of a wild-type animal. The segments shown are T2, T3 and part of A1; arrowheads indicate the anterior margins of these three segments. The thoracic segments have, anteriorly, two or three rows of very short thick hairs that are different in A1. Also, there is a large naked space between T2/T3 and T3/A1, which is characteristic of thoracic segments.

(B) Dorsal view of a *fused*$^-$ embryo from a cross $fu^{1A4}/y\,wffu^{1} \times y\,wffu^{1}$. All the segments are smaller than wild type; arrowheads indicate the anterior margins of T2, T3 and A1 respectively. Notice the reduction of the naked space between T2/T3 and its virtual absence between T3 and A1. On the left-hand side, T3 and A1 appear as if they were fused whereas on the right-hand side, there is still some naked cuticle. A few duplications and polarity reversals can be observed, but they are not the main phenotypic feature.

(C) Ventral aspect of two segments from a wild-type animal : second and third abdominal segments (A2, A3). Notice that different rows of denticles have different polarities (Lohs-Schardin *et al.* 1979). The first (arrows) and fourth rows point anteriorly, while the other rows point posteriorly. Notice the different size of the denticles in the different rows. On the side (arrowhead), notice the sense organ that can be used as a reference for the naked area of the anterior compartment very close to the A/P boundary in the abdominal segments, based on its position in the thoracic segments (Lohs-Schardin *et al.* 1979).

(D) Ventrolateral view of a strong *fused*^{1} embryo. The segments shown correspond to A3 and A4. The first row of denticles (thin arrow) is very distorted. The lateral sense organ (arrows) appears duplicated in A4. Notice that most of the new denticles with reversed polarity lie anterior to the sense organ. Magnifications A,B × 200; C.D. × 320.

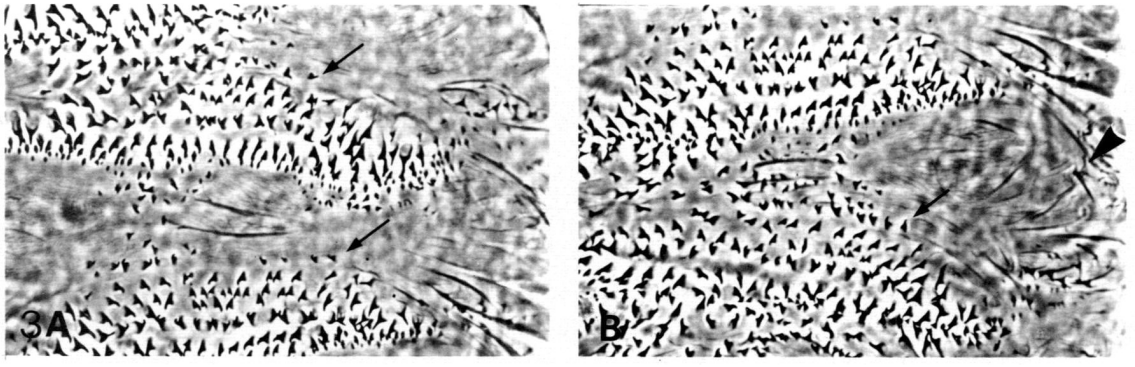

Figure 3. Ventral aspects of segments from homozygous *fused*$^-$ embryos.
(A) The left-hand side is almost wild-type in size and contains a few small and scattered denticles. The right-hand side of this segment is slightly shorter and in this case, it is possible to observe a few extra denticles immediately posterior to the wild-type rows. Arrows indicate the wild-type first row of denticles.
(B) Notice that while the right-hand side shows a pattern similar to the one shown in (A), the left-hand side shows a pattern characteristic of null phenotypes (see Fig. 1) i.e. a large pattern deletion and a substantial number of large denticles with reversed polarity adjacent to the rows of wild-type polarity. Small arrow as in A; arrowhead, lateral sense organ. Magnifications: × 320.

abdominal segments. In spite of this, it is possible to detect the sense organs associated with some of the gnathal segments, in particular the antennal and maxillary sense organs. At their posterior end, *fused*$^-$ embryos have anal pads and posterior spiracles indistinguishable from the wild type (Fig. 1).

The phenotype described above is characteristic of *fused*$^-$ larvae with hypomorphic alleles. A stronger phenotype, i.e. larger deletions dorsally and ventrally, is observed in *fused*$^-$ embryos derived from mothers carrying any allele over a deficiency for the locus, such as fu^{1A4} (Fig. 1).

(b) Embryogenesis of fused$^-$ *embryos*

What follows is an account of the embryonic development of *fused*$^-$ zygotes derived from *fused*$^-$ mothers (see Materials and Methods for genotypes), compared to wild-type embryos.

(i) *Blastoderm formation*

At least one third of the eggs laid by *fused*$^-$ females do not develop; some are not fertilized and some stop cleavage after a few divisions. The other two thirds proceed to form a normal cellular blastoderm. The size of these eggs varies, from an average wild-type size (150 μm × 450 μm) to eggs half this size. Many of these eggs have very little yolk.

(ii) *Gastrulation and germ band extension*

It has been reported that *fused*$^-$ embryos gastrulate abnormally (Counce, 1956). I have found that with the alleles and techniques used, most of the zygotes which develop, gastrulate normally (Fig. 4A). The few exceptions (5 %) display a wide

variety of abnormalities which arrest their development at this stage. These abnormalities include the appearance of secondary cephalic furrows, abundant cell death, or mitotic arrest. After gastrulation, germ band extension takes place normally, together with the early neuroblast segregation.

(iii) *Segmentation and growth*

After germ-band extension, the wild-type embryo enters a phase of growth. At about 5½ h, all derivatives of the different germ layers start to divide asynchronously and the mesoderm undergoes a transient segmentation. It is around this moment that the first deviation from wild-type development is observed in *fused*$^-$ embryos: cells along the germ band appear loose and chromatic droplets (Wigglesworth, 1942) can be observed first in the cephalic mesoderm and shortly afterwards in posterior mesodermal regions (Fig. 4B). These droplets, by their morphology and structure in the electron microscope, are dead cells (Fig. 4). By the time that it is possible to identify the dead cells, their precise origin is difficult to assess since they have been separated from the rest of the cells and they have formed a monolayer between the germ band and the yolk sac. (Figs. 4B).

In the wild type, after the transient mesodermal segmentation, epidermal grooves can be observed that will define metameric units in the embryo, shortly afterwards, the tracheal pits form. In *fused*$^-$ embryos, these processes take place normally. During the growth period that follows and ends at germ band shortening, extensive cell death can be observed in the mesoderm and the ectoderm (Fig. 5A) of *fused*$^-$ embryos. During this second major phase of cell death, there is a cluster of dead cells per metameric unit. Cell death is not observed in the endodermal derivatives: anterior and posterior midgut, which develop normally during this growth period. In *fused*$^-$ embryos, the proctodeal invagination forms normally and pole cells migrate through the posterior midgut (PMG) and organize themselves along the remaining mesoderm of abdominal segments 5 through 9, much in a wild-type manner.

The development of the gnathal segments in *fused*$^-$ embryos parallels that of thoracic and abdominal ones. Excess cell death can be observed in these

Figure 4. Sections through *fused*$^-$ embryos of different ages. All embryos were derived from a cross : $y\,wffu^{10c}/y\,wffu^{1} \times y\,wffu^{1}$.

(A) Horizontal section through the central region of a gastrulating embryo.

(B) Sagittal section through an embryo approximately 5 h old. The cells appear rather loose along the germ band. Notice the clumps of dark cells in the innermost part of the embryo; they are mesodermal dead cells. Bar equals 100 μm.

(C) Higher magnification of the region of cell death. The dead cells can be distinguished from the yolk, in that they have a darkly staining nucleus and a cytoplasm which, at times, is filled with chromatic granules.

(D) Higher magnification through the ventral region of a 7 h-old *fused*^{1} embryo. Notice the looseness of the cells reflected in the spaces between them. Bar equals 10 μm. Abbreviations: *amg*, anterior midgut; *cf*, cephalic furrow; *dc*, dead cells; *ec*, ectoderm; *ms*, mesoderm; *nb*, neuroblasts; *pc*, pole cells; *pmg*, posterior midgut; *st*, stomodeum; *yk*, yolk.

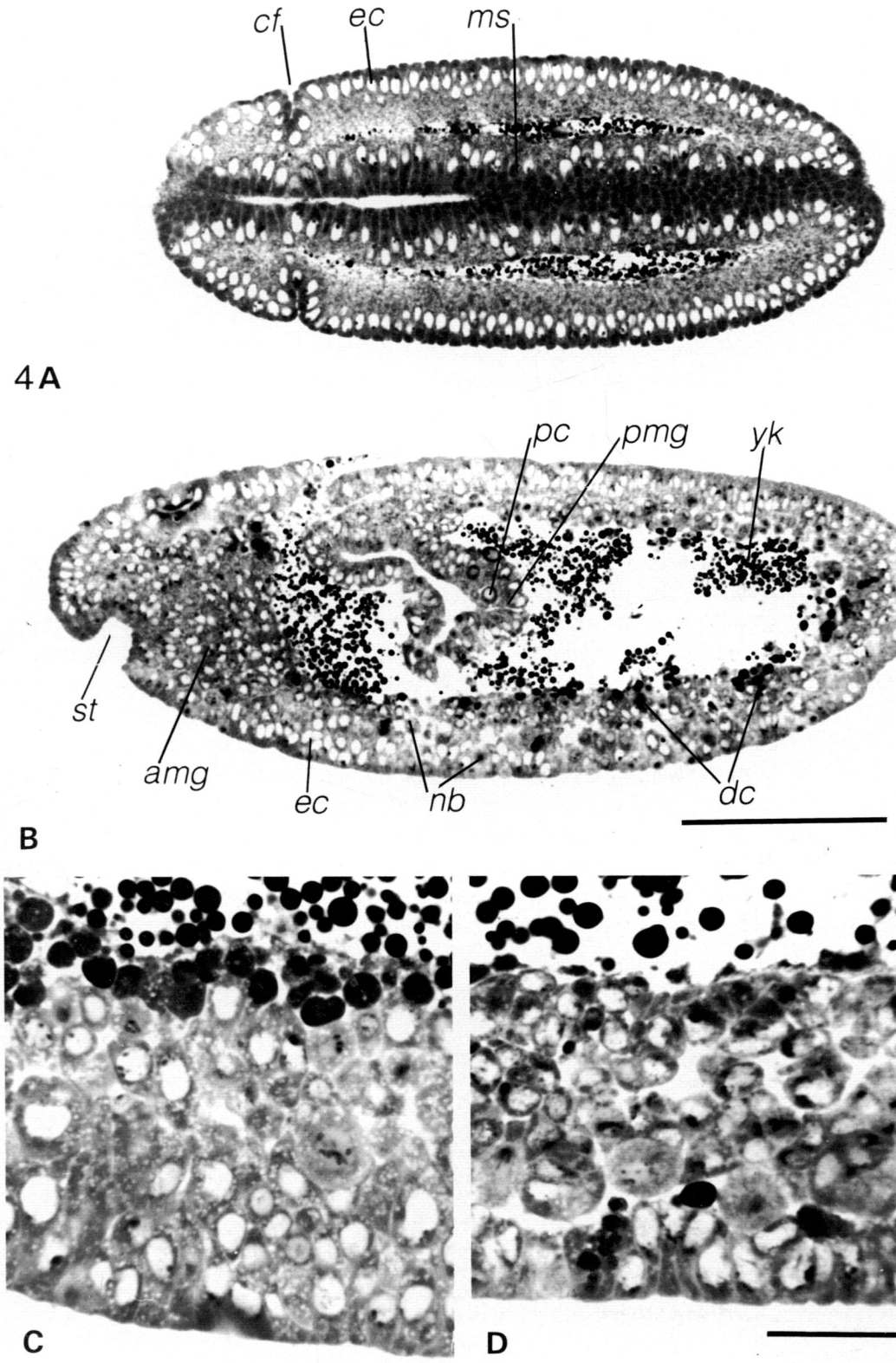

Fig. 4

segments as well as in the cephalic region, where no segmental pattern can be observed in wild type. The salivary glands form normally as derivatives of the labial segment and the invaginations of the roof of the stomodeum that give rise to the stomatogastric nervous system appear at the wild-type place in *fused*$^-$ embryos (Counce, 1956 and pers. obs.).

(iv) *Germ band shortening*

At around 9 h of development, *fused*$^-$ and wild-type embryos undergo germ band shortening. There are three important differences between *fused*$^-$ and wild-type embryos at this stage. The first one concerns the larger number of dead cells that, in *fused*$^-$ embryos, are floating in spaces between germ layers (Fig. 5B). During the process of germ band shortening, all these cells — most of them already phagocytized by macrophages — are placed in a space between the incipient ventral cord and the incipient epidermis, where they are slowly digested. The second difference is the occasional pairwise fusion of the segmental units visible at this stage. These fusions take place more often in the most posterior segments. Finally, at this stage, *fused*$^-$ embryos often show a discontinuous ventral cord; these breaks are, probably, a consequence of the cell death in earlier stages.

(v) *Organogenesis*

From 9½ to 20 h, the wild-type embryo completes development. During this time there are no further cell divisions except in the nervous system (Campos-Ortega, 1982) and towards the end of development among the pole cells (Sonnenblick, 1950). In this period, development of *fused*$^-$ embryos follows the wild type surprisingly closely.

(1) *Mesodermal derivatives:* After germ band shortening, the visceral mesoderm is in close contact with the midgut rudiments. During the next few hours, these cells form the longitudinal and transverse muscles fibres that are responsible for the movement of the gut during embryogenesis and larval development. Myoblasts from the somatic mesoderm start fusing to form first the fibres and then the apodemes when they contact the epidermal muscle attachment sites. These processes of muscle formation take place in *fused*$^-$ embryos in a very similar manner to wild-type embryos. In the 20 h-old *fused*$^-$ embryo it is interesting to note

Figure 5. Sections through *fused*^{1} embryos of different ages. Their genotypes are the same as those shown in Fig. 4.

(A) Lateral sagittal section of an 7 h to 8 h-old embryo. Dead cells are accumulating in intercellular spaces ventrally.

(B) Sagittal section through an embryo after germ band shortening. The dead cells have been phagocytosed by the macrophages and now are in ventral positions.

(C) Dorsal horizontal section of a 20 h-old embryo. Notice how the muscle fibres have formed and attached. The gonads are wrapped by mesoderm and the gut is in the process of completing its constriction. Segments are shorter than wild type. Bar equals 100 μm. Abbreviations: *as*, amnioserosa; *dc*, dead cells; *ep*, epidermis; *fb*, fat body; *go*, gonads; *hg*, hindgut; *mg*, midgut; *ms*, mesoderm; *mf*, macrophages; *mg*, midgut; *oe*, oesophagus; *ps*, posterior spiracle; *sg*, salivary gland; *sm*, somatic muscles; *sp*, supraoesophageal ganglion; *tp*, tracheal pit; *vc*, ventral cord.

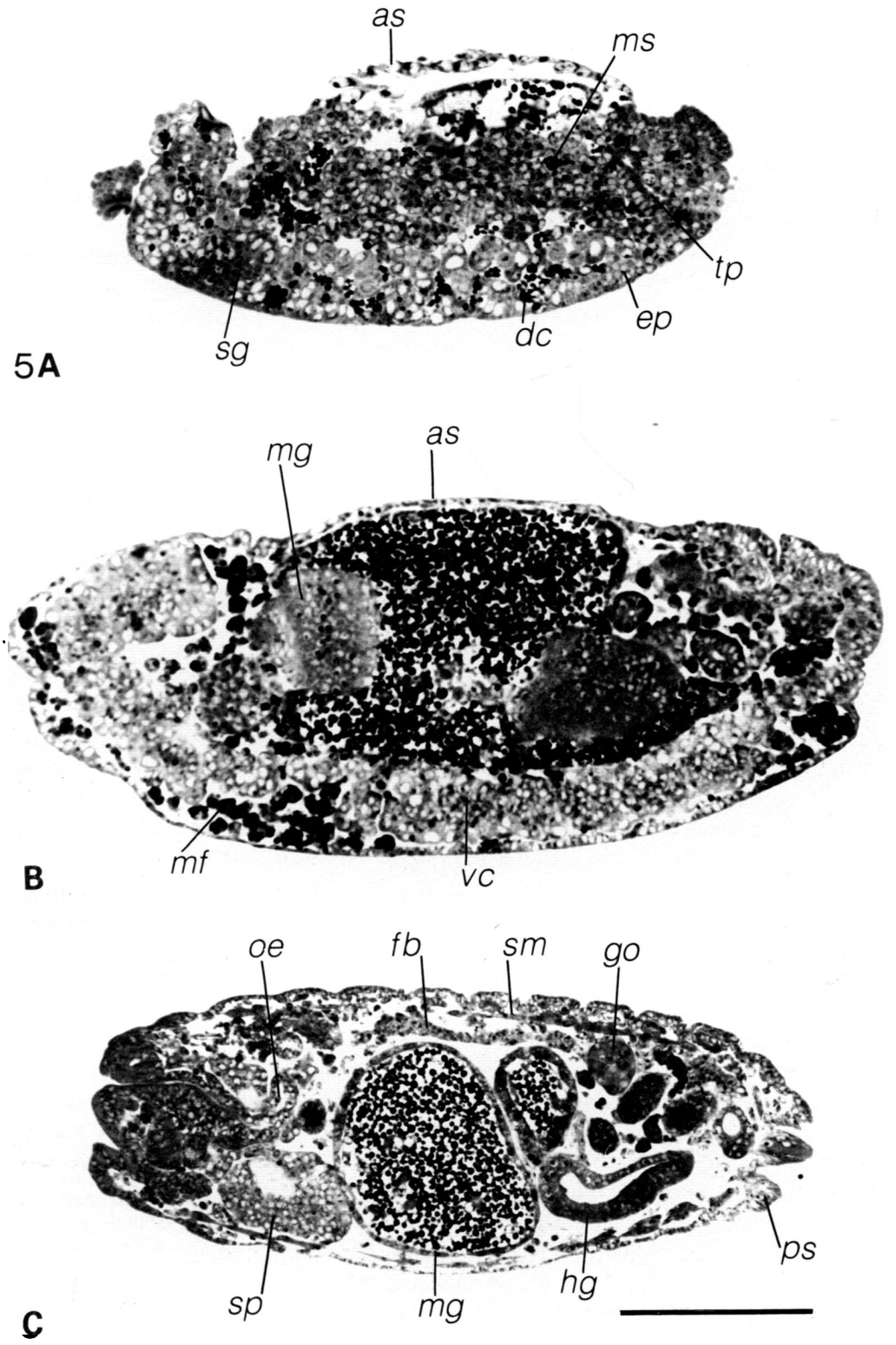

Fig. 5

how the gut has been normally constricted and most of the inter- and intrasegmental muscles formed (Fig. 5C). All this, in spite of the large number of mesodermal cells that died between 6 and 8 h. The gonadal mesoderm and fat body also develop normally (Fig. 5C).

(2) *Ectodermal derivatives:* At around 14 h *fused*$^-$ embryos have segment boundaries as ectodermal grooves determined by the attachment of muscles which are indistinguishable from wild type (Fig. 5C). Salivary glands, malpighian tubules, tracheal pits and subsequently the tracheal tree, all form in mutant embryos.

After germ band shortening, the nervous system of *fused*$^-$ embryos assembles to form a ventral cord that, by 11 h is not distinguishable from wild type.

(3) *Endodermal derivatives:* The anterior and posterior midguts meet and fuse normally in *fused*$^-$ embryos. From this point onwards, although somewhat delayed, the development of the gut proceeds normally. The invaginations that start constricting the gut form and the cardia will arise later at the normal location and with normal structure.

(4) *The head: Fused*$^-$ embryos start and complete the process of head involution in an almost wild-type manner; differences are due to the adaptations required by the shorter gnathal and cephalic segments of *fused*$^-$ embryos. In the mature embryo, the antennal and maxillary sense organs, as well as the mouth hooks occupy wild-type positions, supporting the notion that head involution takes place in a wild-type manner.

DISCUSSION

Pattern duplications are well-known phenomena in biological systems (Bateson, 1896). They arise either as a response of the system to mechanical damage (Bohn, 1970; Lawrence, 1973; Wright & Lawrence, 1981) or as alternative developmental pathways in animals bearing certain mutations (Nüsslein-Volhard *et al.*, 1982). Duplications are, often, associated with the loss of pattern elements so that they are substitutions of a part of the pattern by a copy of another part. In general they are interpreted on the basis of regulative properties of gradients that underlie pattern (reviewed in Lawrence, 1973) or as the outcome of a compensatory computation of positional information (French, Bryant & Bryant, 1976; Wright & Lawrence, 1981; reviewed in Lawrence, 1981).

Mutations at the *fused* locus of *Drosophila* affect the pattern in a manner reminiscent of duplications: a part of the segment is missing and the residual posterior region displays anterior pattern elements with a reversed polarity. Here the deviations from wild type that take place during the embryonic development of *fused*$^-$ embryos will be discussed. These deviations will be related to the final cuticular pattern in an attempt to understand the wild-type function for this locus.

(a) *The origin of the pattern duplication*

Ventrally, in the wild-type first-instar larva, the anterior compartment of each segment consists of two regions: an anterior one covered with denticles and a

posterior one between the last row of denticles and the A/P compartment boundary, which is naked; the posterior compartment is completely naked (Figs 1 and 2). This description relies, in the thorax, on the relationship between the Keilin's organs and the A/P compartment boundary (Struhl, 1984). In the abdomen a similar landmark is not available. On the side of every segment, there is a sense organ which, in the thoracic segments, can be shown to lie immediately anterior to the compartment boundary, within the region of clear cuticle of the anterior compartment (Hertweck, 1931; Lohs-Schardin *et al.* 1979). This sense organ is an anterior landmark that is close to the anteroposterior compartment boundary in the abdomen as well as in the thorax (Fig. 2). In embryos carrying weak alleles of *fused* and in some segments of embryos derived from females of different alleles over a deficiency for the locus, this sense organ is present. In these cases, most of the denticles with reversed polarity lie anterior to the sense organ (Figs 2 and 3). An observation which suggests that these denticles are produced mainly by cells within the anterior compartment and that the deleted region is, mostly, from the posterior compartment. However, the absence of the pattern elements that define the compartment boundary (Keilin's organs in the thoracic segments) suggests that some cells from the anterior compartment are also eliminated.

The pattern duplications observed in *fused*$^-$ embryos seem to be related to the pattern deletions. In larvae bearing weak alleles, it is possible to observe that the bigger the deletion, the larger the duplication (Figs. 1 and 3), which suggests that the polarity reversal may be a secondary consequence of the deletion. This observation resembles a result by Wright & Lawrence (1981): after removal of a large fragment of the positional field between two segments of the insect *Oncopeltus*, they obtained reversal of polarity by intercalation, and also induced a reversal of polarity in the remaining most posterior part of the segment (see their experiment 2 and fig. 9 in Wright & Lawrence, 1981). In both cases, the contact between very distant positional values induces a polarity change.

The absence of Keilin's organs suggests that the normal A/P compartment boundary is not present in *fused*$^-$ embryos; this, however, does not imply the absence of any A/P boundary. The criterion whereby the A/P boundary is defined is a lineage one and implies an interface between cells with different genetic addresses (Garcia-Bellido, Lawrence, & Morata, 1979). In *fused*$^-$ embryos there are anterior and posterior cells that probably form an interface. Only lineage experiments with appropriate markers would reveal this secondary A/P boundary.

An intriguing difference exists between the dorsal and the ventral surfaces of *fused*$^-$ embryos: pattern deletions can be observed dorsally and ventrally, but, while ventrally pattern duplications are associated with the deletion, dorsally there is no substantial pattern substitution or polarity change among the residual elements. Dorsally, the major effect of the *fused* mutation is the deletion of the pattern. The fact that the dorsal phenotype is more obvious in embryos derived from mothers heterozygous for a deficiency suggests, however, that in embryos with true null alleles polarity changes could be observed dorsally.

(b) *The embryonic development: cell death*

The development of *fused*$^-$ embryos cannot be distinguished from wild type until approximately 5 h of development, when cell death can be observed in the mesoderm from cephalic to caudal positions. It is interesting that the first dead cells appear in the mesoderm at a time when this germ layer undergoes a transient morphological segmentation. The second phase of cell death takes place at the core of the growth period when, presumably, the cells start "realizing" the segmental commitments adopted at around blastoderm. In this context, it is noticeable that no abnormalities are observed in endodermal derivatives. Since the *fused*$^+$ locus seems to be involved in some aspect of segmentation, this observation suggests that the endodermal derivatives are not segmented.

Why do cells die? The fact that cells die at specific times and at specific places due to particular mutations has been observed before in *Drosophila* (Fristrom, 1969) but no explanation has been put forward. Cells might die because, for instance, they fail to receive a trophic factor that is required by them at a given time to perform a function. It is possible to argue that the gradient responsible, among other things, for the final epidermal pattern, is a gradient of such a trophic factor and that, somehow, it is altered locally at the place of cell death. A second possibility is that cells die because they fail to communicate with their neighbours. In this regard, it is interesting that in *fused*$^-$ embryos, at around the time of cell death, cells of different germ layers appear rather loose (Fig. 4B), as if there were failures in cell–cell contacts.

Is there a cellular turnover to replace the dead cells? This is particularly important since regeneration has been observed to produce pattern duplications similar to those noticed in *fused*$^-$ embryos (Wright & Lawrence, 1981). After germ band shortening, no extra cell divisions are observed in morphological preparations in the mesoderm or the epidermis. However, if extra cell divisions took place at the same time that the cells are dying, it would be very difficult to detect them since, at that time, the whole embryo is engaged in an active programme of cell division. The fact that the terminal *fused*$^-$ embryo is much smaller than wild type, suggests that if there is regeneration, it is very limited. In this respect, experiments removing small numbers of cells from blastoderms in different manners (Lohs-Schardin *et al.* 1979; Underwood, Turner & Mahowald, 1980) have never detected pattern duplications or rearrangements but only the absence, in the mature embryo, of the part ablated at blastoderm. These results suggest that the regenerative abilities of embryos are very limited and that in *fused*$^-$ embryos there is likely to be no regeneration.

(c) *A wild-type function for* fused$^+$

The main features of the *fused*$^-$ phenotype can be summarized as follows:

(i) In *fused*$^-$ larvae, part of every segment is absent dorsally and ventrally and part of the remaining pattern is duplicated.

(ii) Extensive cell death occurs within each segment during embryogenesis. The absence of these cells in the embryo is likely to account for the pattern deletions observed in the larvae.

(iii) The pattern duplications observed can be interpreted as a secondary consequence of the deletions. It appears that the larger the deletion, the larger the duplication.

These observations suggest that the *fused*$^+$ product is required in a certain region of each segment. Absence of the product leads to cell death. The region where *fused*$^+$ is required must lie around the anteroposterior compartment boundary, since pattern elements of this region are absent even in animals with small pattern deletions and thus, few duplications.

The embryonic development of *fused*$^-$ embryos indicates that *fused*$^+$ is not required for the determination or the establishment of segmental primordia, since the embryological processes related to these events take place normally in mutant embryos. Cell death is observed in these embryos at the time metameric units become morphologically defined. It is thus possible that *fused*$^+$ is required in the embryo for late steps in the organization of segmental units.

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