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Developmental Analysis of the Torso-like Phenotype in *Drosophila* Produced by a Maternal-Effect Locus

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The segmental plan of the *Drosophila* embryo is already established at the blastoderm stage through the action of maternal effect genes which determine the polarity of the embryo and zygotically active genes involved in segmentation. We have analyzed the first example of a group of maternally acting genes which are necessary for establishing the developmental potential of the posterior 25% of the blastoderm. Females, homozygous for the X-linked maternal-effect mutation *female sterile(1)Nasrat*^{211} [*fs(1)N*^{211}], produce embryos, characterized as torso-like, which lack all posterior endodermal derivatives as well as structures characteristic of abdominal segments 8 to 10. In addition, anterior endodermal derivatives are deficient and the absence of pharyngeal musculature causes a collapse of the cephalopharyngeal apparatus. The columnar blastoderm cell layer is defective at the posterior tip below the pole cells in these embryos. This defect, however, is presumably secondary to some abnormal feature of pole cell formation since in double mutants of *fs(1)Nasrat*^{211}; tudor^3 the blastoderm is normal but the embryos still show the torso-like phenotype. *In situ* hybridization with RNA probes derived from the *fushi tarazu* gene establishes that the cellular determination of the posterior blastoderm of embryos produced by *fs(1)N*^{211} is changed. This represents the first direct demonstration that a maternal-effect mutation alters the spatial distribution of a zygotic gene product involved in the segmental patterning of the embryo.

INTRODUCTION

The animal oocyte provides the cellular environment in which the processes of embryogenesis are set in motion at fertilization. Initially, the zygotic genome is inactive, the duration of the inactivity depending upon the organism (see Davidson (1976) for review). In *Drosophila melanogaster* approximately the first 1½ hr of embryonic development are directed solely by RNA transcribed during oogenesis (Zalokar, 1976; Anderson and Lengyel, 1980; Steller and Pirrotta, 1984), followed by a period of combined zygotic gene expression and perdurance of the maternal contribution (reviewed in Mahowald and Hardy, 1985). Recent systematic approaches utilizing saturation mutagenesis screens have begun to clarify the respective roles of maternally and zygotically expressed gene functions in embryogenesis. Thus, many maternal effect mutations appear to define functions involved in setting up the major axes of the embryo (Anderson and Nusslein-Volhard, 1984). Zygotic gene functions in combination with maternal functions (Perrimon and Mahowald, 1986) establish the segmental pattern and identity.

Commitment of cells to specific developmental fates appears to be established soon after their formation at the blastoderm stage. This has allowed the construction of fate maps showing the relative position of segment primordia at the blastoderm stage (Lohs-Schardin *et al.*, 1979; Underwood *et al.*, 1980). Recently, direct molecular evidence for qualitative differences between blastoderm nuclei has been presented. Thus the *fushi tarazu* (*ftz*) gene, which is known to be required for the formation of the correct number of segments, is expressed in a spatially specific fashion even prior to cellularization (Hafen *et al.*, 1984). The overall anterior–posterior segmental pattern of the embryo is thought to be controlled by maternal effect genes (Nusslein-Volhard, 1979). It has therefore been suggested that maternal effect genes might be responsible for the pattern of expression of the *ftz*$^+$ product (Hafen *et al.*, 1984).

Two sets of maternal effect lethal mutations have been recovered which appear to perturb specifically differentiation of the posterior region of the embryo. The prototypes of these two classes have been termed *tudor* and *torso* (Nusslein-Volhard *et al.*, 1982). In tudor-like mutations, the posterior germ plasm fails to form and the abdominal segments anterior to the eighth are either absent or abnormal (Boswell and Mahowald, 1985). In torso-like mutants, both the posterior gut derivatives and the posterior-most abdominal segments fail to develop; in addition, some anterior components are abnormal.

In this paper we provide a detailed developmental analysis of the torso-like phenotype produced by

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fs(1)N^{211}, a mutant allele of the X-linked locus *fs(1)N* and report on its interactions with a *tudor* allele.

MATERIALS AND METHODS

An allele of *fs(1)Nasrat* (Counce and Ede, 1957) was obtained during an ethyl methane sulfonate (EMS) mutagenesis screen of the X-chromosome for maternal effect lethal mutations (Engstrom and Mahowald, unpublished). This mutation, called *fs(1)N*^{211} also fails to complement the *M6* class of female sterile mutations in the Mohler screen (Mohler, 1977) and *A371* in the Gans collection (Gans *et al.*, 1975). The locus is uncovered by *Df(1)A94*, *Df(1)S39* and *Df(1)sta*, establishing its location in the 2A-B region of the X-chromosome (Kern, 1977). In the homozygous configuration and in trans heterozygous combinations *inter se*, the Gans and Mohler alleles produce flaccid eggs which collapse prior to cuticle differentiation. Most of the eggs produced by homozygous *fs(1)N* females also are flaccid, but in addition they produce a class of late embryonic lethals (Counce and Ede, 1957) which have a cuticle phenotype similar to that produced by homozygous *fs(1)N*^{211} mothers (Degelmann, unpublished results). Interestingly, in trans with *fs(1)N*^{211}, all the other alleles tested result in the *fs(1)N*^{211} phenotype. A more extensive genetic analysis of this locus will be presented elsewhere.

Embryos were examined by a variety of procedures. Embryos of *fs(1)N*^{211} homozygous females were collected at 25°C on agar plates and aged for the appropriate time. For scanning electron microscopy, embryos were prepared according to Turner and Mahowald (1976); for transmission electron microscopy, embryos were fixed in glutaraldehyde and formaldehyde (Kalt and Tandler, 1971), postfixed in 1% osmium tetroxide, stained in 0.5% uranyl acetate overnight, and embedded in Dow epoxy resin (Mahowald *et al.*, 1979). For thick sections, chorions and vitelline membranes were removed with 2.5% sodium hypochlorite and a modification (Dequin *et al.*, 1984) of the Mitchison and Sedat (1983) procedure, respectively. Fixation was either according to Zalokar and Erk (1977) or Dequin *et al.* (1984). After post-fixation in glutaraldehyde–formaldehyde (Kalt and Tandler, 1971), embryos were embedded in plastic and sectioned at either 1 or 3 μm, and stained with Toluidine blue at pH 10.5. For horseradish peroxidase (HRP) antibody staining, embryos were treated as in Dequin *et al.* (1984). They were incubated in a 1:50 dilution of FITC-labeled rabbit anti-HRP (Cappel) in phosphate-buffered saline (PBS) + 1% bovine serum albumin (BSA) at 25°C overnight. After extensive rinsing in PBS + 1% BSA, embryos were mounted in 90% glycerol and observed under epifluorescent illumination. Embryonic cuticle preparations were prepared after van der Meer (1977).

In situ hybridization to embryo sections. For synthesis of the *ftz* probe for *in situ* hybridization, we used the plasmid pSF1, which was constructed in Walter Gehring's laboratory. pSF1 contains the 1.1-kb *ftz* cDNA (Kuroiwa *et al.*, 1984) in antisense orientation with respect to the SP6 promoter of pSP65 (Melton *et al.*, 1984). Full length antisense RNA, labeled with [^{3}H]UTP was transcribed with SP6-polymerase (Promega Biotec) using the protocol provided by the supplier, which is based on the assay conditions of Melton *et al.* (1984). After transcription, the [^{3}H]RNA was subjected to a limited alkaline hydrolysis to obtain a main fragment length of 150 bases (Cox *et al.*, 1984).

Both *fs(1)N*^{211} and wild-type embryos, aged to 2–4 hr, were prepared according to Dequin *et al.* (1984). Embryos, without vitelline membranes, were rehydrated through 90, 70, 50, and 25% methanol in phosphate-buffered saline (PBS) and post-fixed in 4% paraformaldehyde in PBS (Akam and Martinez-Arias, 1985). After rinsing in PBS, the embryos were embedded in O.C.T. (Miles), frozen in liquid nitrogen and 8 μm frozen sections collected on subbed slides. Pretreatment of the sections for *in situ* hybridization was carried out according to Hafen *et al.* (1983), with an acetylation step (Hayashi *et al.*, 1978) after the pronase digestion. Hybridization and washing of the sections was performed after Knipple *et al.*, (1985). The sections were hybridized in 50% formamide, 0.6 *M* NaCl, 10 m*M* Tris-HCl (pH 7.6), 1 m*M* EDTA, 1× Denhardt's solution, 0.5 mg/ml tRNA, and 0.5–1 μg/ml *ftz*-RNA for 24 hr at 44°C. Following digestion with 20 μg/ml RNase A, the slides were washed in large volumes of 4× SSC and 2× SSC for several hours each. After dehydration and drying the slides were coated with Kodak NTB-2 emulsion and exposed at 4°C for 10 to 30 days.

RESULTS

Cuticular Morphology of Embryos from fs(1)N^{211} *Females*

The *fs(1)N*^{211} mutation was induced in a wild-type Oregon R P2 stock. Some of the work described in this paper was carried out with this mutation recombined to an X-chromosome bearing *white*, *miniature*, and *split*. The most striking feature of the cuticle pattern of the mutant is the presence of only six complete abdominal denticle belts instead of the usual eight (Fig. 1). In embryos produced by flies homozygous for the mutation on the original chromosome or heterozygous over a deficiency for the locus, there is a terminal cluster of setae presumed to represent part of the seventh abdominal segment. In embryos produced from flies homozygous for the recombined chromosome, occasionally a full seventh denticle belt is present and in rare instances partial

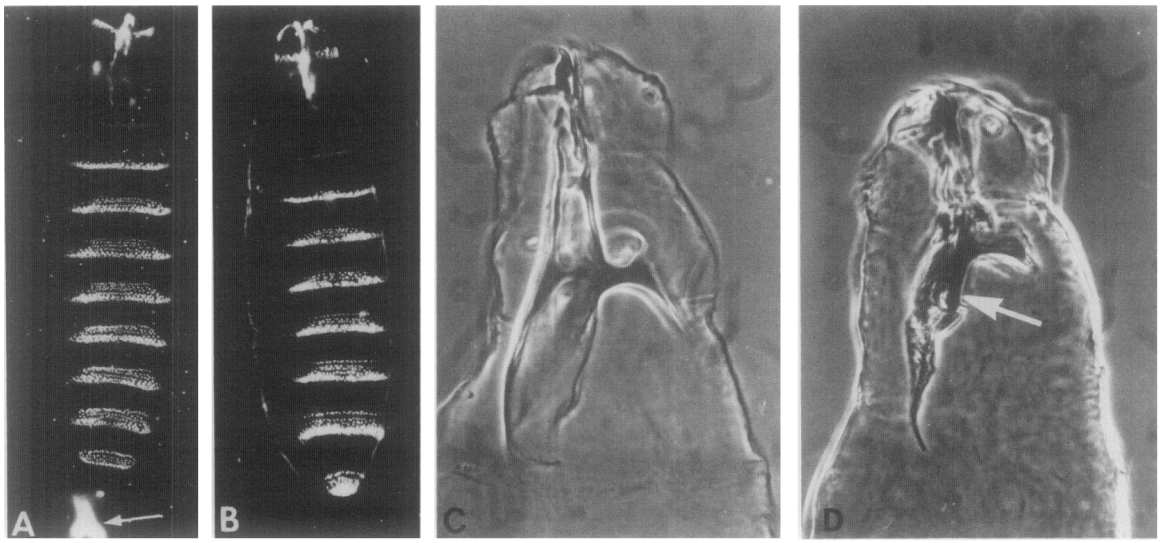

FIG. 1. Cuticle preparations of wild-type (A, C) and *fs(1)N*^{211} (B, D) embryos. The typical eight ventral abdominal denticle belts are found in wild-type (A) but only six belts and distal tuft are seen in the mutant (B). The Filzkorper (arrow) and spiracles are present in wild-type but not the mutant. The mouth parts are shown at higher magnification in (B) and (D). The major difference is the collapse of the pharyngeal apparatus (arrows) around the esophagus. (A, B: ×60; C, D: ×200)

eighth belts. However, there are never any dorsal eighth abdominal structures such as Filzkorper or spiracles. The anus, anal pads, and anal tuft are likewise absent. The lateral tracheal trunks end blindly, usually in the sixth abdominal segment. Defects of varying severity are also noted at the anterior end. In some embryos, the head segments fail to involute and only sclerotinized fragments of the cephalopharyngeal apparatus (CPA) are found. In most cases, however, head involution is apparently complete but the internalized CPA lacks the median tooth and appears collapsed (Figs. 1, 2). The maxillary-antennal sense organs and cirri are found after head involution but are not visible in those embryos with external chitinous plates. This combination of phenotypic features of *fs(1)N*^{211} resembles those described for *torso* (Nusslein-Volhard *et al.*, 1982). In addition, it corresponds closely to the cuticle phenotype of the late embryonic lethal class produced by the original *fs(1)N* allele (Counce and Ede, 1952; Degelmann, unpublished results).

Scanning Electron Microscopy of Torso-like Embryos

A survey of *fs(1)N*^{211} embryos by scanning electron microscopy clearly suggested that there was an absence of all structures normally derived from the posterior 25% of the blastoderm fate map (cf. Hartenstein *et al.* (1985) for fate map). Thus, at the time of gastrulation, neither the posterior midgut invagination nor the proctodeal invagination appears (Fig. 2A). While germ band elongation occurs normally, it is clear that at the first appearance of segmentation only seven abdominal segments form (Fig. 2B). Thus, the absence of these posterior derivatives can be detected at very early stages of gastrulation and segmentation. The defect responsible for the aberrant anterior development is not as clear. In some instances the stomodeal invagination is unusually small (Fig. 2C), suggesting that defects in this invagination may cause failure of head involution. However, in most individuals, head involution is normal.

Histological Analysis of Torso-like Embryos

We have undertaken a detailed histological examination of developing embryos to investigate the development of internal organ systems in *fs(1)N*^{211} embryos. Posteriorly, the posterior midgut and hindgut are totally missing, as would be expected due to the absence of their primordia at gastrulation. The Malpighian tubules, which are normally found at the junction between the mid- and hindgut, also fail to form. The original yolk sac, which forms as a syncytium at the time of gastrulation (Rickoll and Counce, 1980), becomes cellularized (Fig. 3B). This suggests that a process of membrane furrowing must occur in this tissue to produce the large cells surrounding the yolk. This observation also supports the previous suggestions (Poulson, 1950) that the "primitive yolk cells," as they were called, can participate in the formation of the definitive embryonic midgut.

Extensive pockets of pycnotic cells are observed in the sub-hypoderm region of the posterior abdomen (Fig. 3A) and probably represent components of both the neural and mesodermal derivatives. Cell death is first seen at about 6 hr of development at the tip of the extended

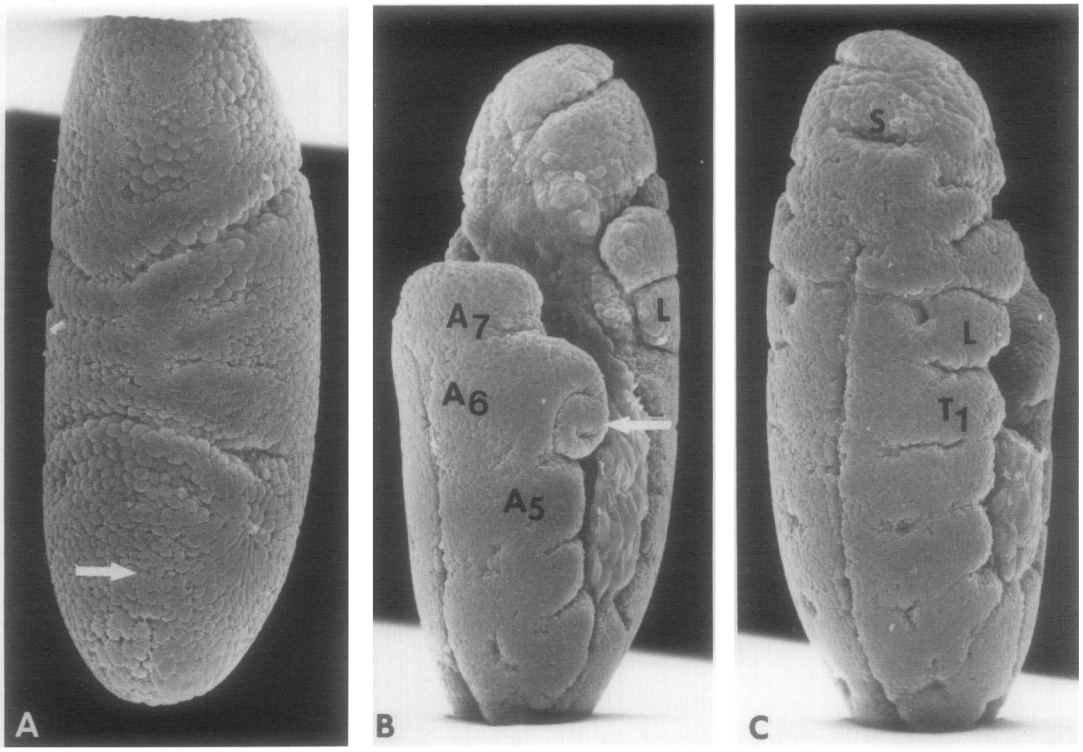

FIG. 2. Scanning electron micrographs of *fs(1)N*^{ts11} embryos (anterior is up in each panel). The absence of the posterior midgut invagination (arrow indicates site where it should be) in the early gastrula stage embryo (A) is clearly evident at 3.5 to 4 hr. Panels B and C show dorsal and ventral views of the same 8-hr embryo. The labial (L) segment followed by three thoracic and five abdominal segments are clearly evident (T_1 to A_5). Abdominal 6 and 7 are less clearly separated. The curl (arrow) on A_6 is unusual. The stomodeum (S) is only a slight invagination at a time in development when it is ordinarily very deep (cf. Turner and Mahowald, 1977). (×270)

germ band and continues as a major feature until after germ band contraction at 12 hr. Although the exact origin of the dying cells is difficult to determine, possibly, both the neural and mesodermal precursors for components of the gut system form normally and then, in the absence of their target organs, degenerate. After staining of the nerve cord with fluorescent anti-horseradish peroxidase antibodies (Jan and Jan, 1982), 10 segmental ganglia can be distinguished, corresponding to the three thoracic and seven abdominal segments formed (Fig. 4). There also appears to be an 11th area of stain present which does not show the typical ladder structure. This may indicate that at least some neurons capable of reacting with the antibody form a cluster posterior to the 7th abdominal ganglion, in spite of the failure to differentiate the 8th segment.

Our histological analysis also throws some light on the defects which underlie the mutant anterior cuticular phenotype. The esophagus is usually complete but only in some embryos does the proventriculus form. The remaining portions of the anterior midgut are always absent. Widespread cell death also occurs anteriorly, presumably among the neural tissue of the procephalic lobe (Fig. 3A). Later, there is little evidence of cell death in the embryonic brain itself and the major areas of cell death are associated with the regions which form the stomatogastric nervous system. This last finding may be related to the absence of normal pharyngeal musculature in those embryos with complete head involution (Fig. 5). The lack of pharyngeal muscle is doubtless responsible for the collapsed appearance of the CPA noted in cuticle mounts.

The Blastoderm Phenotype of Mutant Embryos

In order to pinpoint the earliest deviation from the normal developmental pathway, embryos during blastoderm formation were examined in 1- to 3-μm plastic sections and by electron microscopy of thin sections. The synchronous mitotic divisions of the syncytial stage and blastoderm formation occur normally except at the posterior pole. The initial formation of pole cells appears normal except that a considerable number of polar granules remains associated with the blastoderm layer. Subsequently, there appears to be a second wave of pole cell formation. In addition, many of the pole cells acquire abnormal shapes and, in some instances, appear to be fragmenting. In most individuals, pole cells are lost from

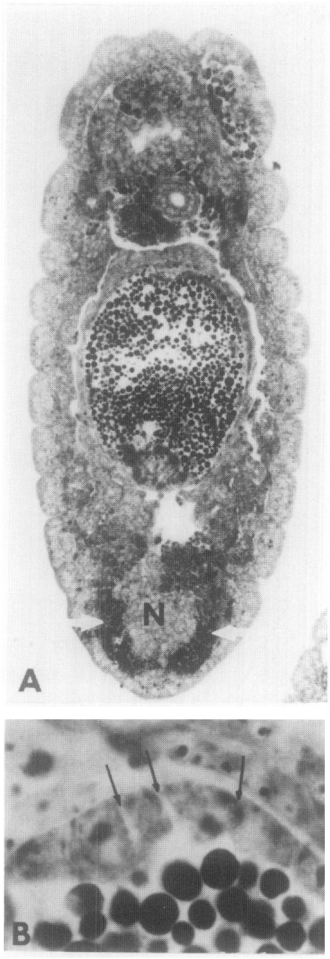

FIG. 3. (A) A frontal view of an 11-hr *fs(1)N*^{211} embryo showing the extensive cell death (arrows) in the fused A_6 and A_7 segments around the posterior end of the vental nerve cord (N) as well as in anterior regions, (×120). (B) Higher magnification of the cellularized yolk sac. The arrows point to the cell membranes. (×1000)

embryos during gastrulation, but occasionally a normal gonad is found suggesting that these pole cells can reach the gonads in some instances even in absence of the posterior midgut invagination.

Following pole cell formation and during cellularization of the blastoderm, a second defect occurs at the posterior tip. Although nuclei are located in normal frequency in the sub-pole cell region of the syncytial blastoderm, they fail to elongate properly (cf. Fullilove and Jacobson, 1971). Subsequently, when furrows have appeared around other blastoderm nuclei, no furrows form around the nuclei at the posterior tip (Fig. 6), giving the impression of a hole in the blastoderm layer. We term this aspect of the mutant phenotype the "pole hole." Ultrastructural analysis of this region demonstrated that the centrosome region, which is usually located at the apex of each nucleus (Huettner, 1933; Mahowald, 1963; Karr and Alberts, 1985), is found at any position around these nuclei (Fig. 7). Moreover, the centrosome region is remarkably free of microtubules as seen either in thin section (Fig. 7) or by immunofluorescence staining with anti-tubulin antibody (data not shown). Some of the posterior nuclei have taken on the appearance of yolk nuclei, as evidenced by their chromatin structure and irregular shape. At the end of blastoderm formation, these nuclei are pushed toward the yolk where they become indistinguishable from yolk nuclei.

Defect in Posterior Blastoderm Cellularization Is Not Required for the Torso Phenotype

Our histological studies demonstrated that pole cell formation is abnormal in *fs(1)N*^{211} embryos. It seemed possible that the abnormal cellularization of the posterior blastoderm was actually caused by the abnormal pole cell formation. To test this hypothesis, we investigated the effects of suppression of pole cell formation on cellularization of the posterior blastoderm. Consequently, we examined embryos produced by females, doubly mutant for *fs(1)N*^{211} and *tudor*^{3}. All embryos derived from females homozygous for the *tudor*^{3} allele lack pole cells. Subsequently, two different phenotypes are seen (Boswell and Mahowald, 1985): 60% of the embryos

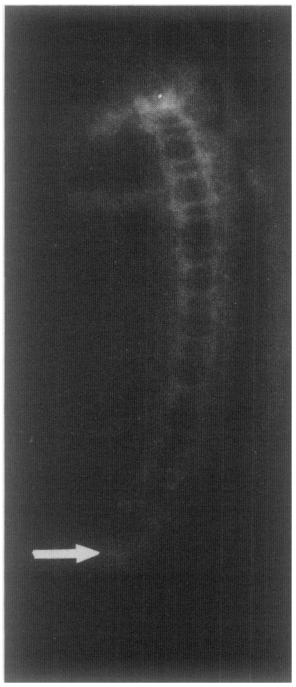

FIG. 4. Whole mount preparation of a *fs(1)N*^{211} embryo, stained with FITC-labeled anti-horseradish peroxidase. The ladder-like structure characteristic of the embryonic nervous system is visible for all segments, including A_7. Some disorganized nervous tissue can be seen posterior to A_7 (arrow). (×140)

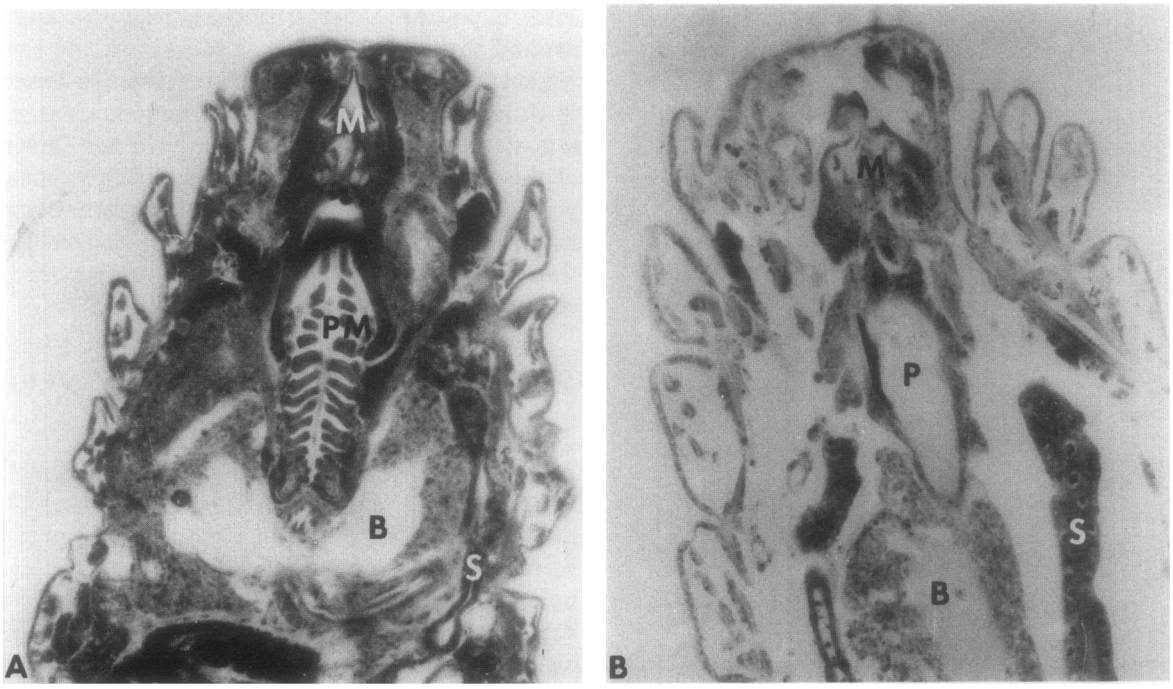

FIG. 5. Frontal sections of wild-type (A) and *fs(1)N*^{211} (B) embryos, showing the structure of the cephalopharyngeal region. Both embryos show the normal disposition of the mouth hooks (M), brain (B) and salivary glands (S). However, while in the wild-type, the pharynx is marked by a characteristic pattern of muscle fibers (PM), the mutant completely lacks musculature in the pharynx (P). (×230)

have normal segmentation, hatch, and grow into sterile adults; the remaining 40% die as embryos and exhibit severe abdominal defects although abdominal 8 with Filzkorper and spiracles are always present.

All progeny derived from double-mutant females fail to hatch. On inspection of cuticle preparations, two classes of embyros are found. One class shows the typical torso-like phenotype and presumably corresponds to that proportion of *tudor* progeny which would have hatched in the absence of the *fs(1)N*^{211} mutation. Moreover, it indicates that the absence of pole cells does not change the phenotype produced by the *fs(1)N*^{211} mutation. The second class of embryos displays the *tudor* cuticular phenotype characterized by fusions of the abdominal denticle belts (Fig. 8). However, these embryos also show a complete absence of the terminal abdominal segments. The absence of the spiracles and other elements of the posterior abdomen clearly indicates that the torso-like phenotype has been superimposed on the tudor phenotype.

Whereas cuticle preparations of mature embryos showed an additive effect of the two mutations, histological analysis of the blastoderm failed to detect any defect in the blastoderm at the posterior tip apart from the absence of pole cells caused by the *tudor*^{3} mutation. Hence, the genetic inhibition of pole cell formation repairs the defect in cellularization of the posterior blastoderm without affecting the expression of the torso-like phenotype of *fs(1)N*^{211}. Consequently, we conclude that the pole-hole defect in the posterior blastoderm is linked to abnormal pole cell formation and is not causally related to the absence of structures derived from the posterior 25% of the blastoderm.

The Expression of a Segment-Specific Gene Is Changed at the Blastoderm Stage

The finding that the *fs(1)N*^{211} mutant cuticle phenotype is still expressed in embryos showing apparently normal posterior blastoderm cells suggests that their state of determination might be abnormal. The recent demonstration of segment-specific expression of the *fushi tarazu* (*ftz*) gene (Hafen *et al.*, 1984) provides an ideal probe to test this possibility. In wild-type embryos, *ftz* RNA is found in seven evenly spaced cellular stripes between 15 and 65% egg length. The stripes are thought to correspond to alternating segmental units, the anteriormost stripe comprising the posterior head segments and the posteriormost stripe corresponding to the "parasegment" consisting of the posterior portion of abdominal 8 (A8p) and the anterior portion of abdominal 9 (A9a) (Martinez-Arias and Lawrence, 1985; Hafen *et al.*, 1984). If the absence of the latter segments in mutant embryos were caused by an alteration in the determination of their primordia at the blastoderm stage, we would expect to find an abnormal pattern of *ftz* RNA expression. We therefore hybridized *ftz* antisense RNA to frozen sec-

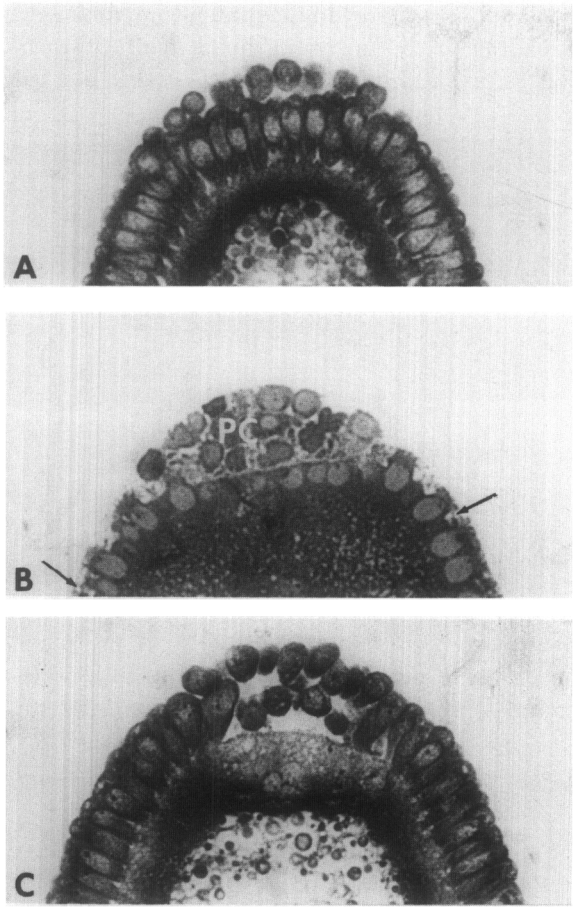

FIG. 6. Light micrograph of an early (B) and mid- (C) blastoderm formation stage of *fs(1)N*^{211} embryos. Although membrane furrows are forming around the lateral blastoderm nuclei (arrows), no furrows form at the posterior tip below the pole cells (PC). The resulting "pole hole" is clearly seen in C. Panel (A) shows the wild type stage corresponding to (C). (×300)

tions of 2–4 hr *fs(1)N*^{211} embryos. Two striking deviations from the wild-type pattern are immediately obvious. First, there are only 6 zones of hybridization, and second, the distance between the 5th and 6th labeled zones is always greater than the distance between the others. In wild-type embryos the zones are evenly spaced (cf. Hafen *et al.*, 1984). In addition, the 6th band is consistently broader than the other 5 bands. At the late blastoderm stage, the space between stripes 5 and 6 is greater ventrally than dorsally (Figs. 8B, C), probably as a consequence of cell movements at the beginning of gastrulation. Measurements of the relative positions of the labeled zones with respect to total egg length suggest that the 5 anterior stripes lie within the average positions mapped by Hafen *et al.* (1984) in wild-type. The anteriormost cluster lies at 65% egg length and can be found in the posterior border of the cephalic furrow at early gastrulation. The sixth stripe appears at about 18% egg length and is thus shifted significantly from its usual position in wild-type embryos. This stripe has been interpreted as representing a "parasegment," consisting of the posterior compartment of abdominal 6 and the anterior compartment of abdominal 7 (Martinez-Arias and Lawrence, 1985). The final abdominal cuticle pattern of *fs(1)N*^{211} embryos (see Fig. 1) supports this interpretation. Thus, the absence of posterior cuticle structures evidently results from an alteration of the blastoderm fate map, as demonstrated by the truncation of the normal *ftz*$^{+}$ pattern.

DISCUSSION

Many studies have suggested that a major function of maternal information is to provide a spatial coordi-

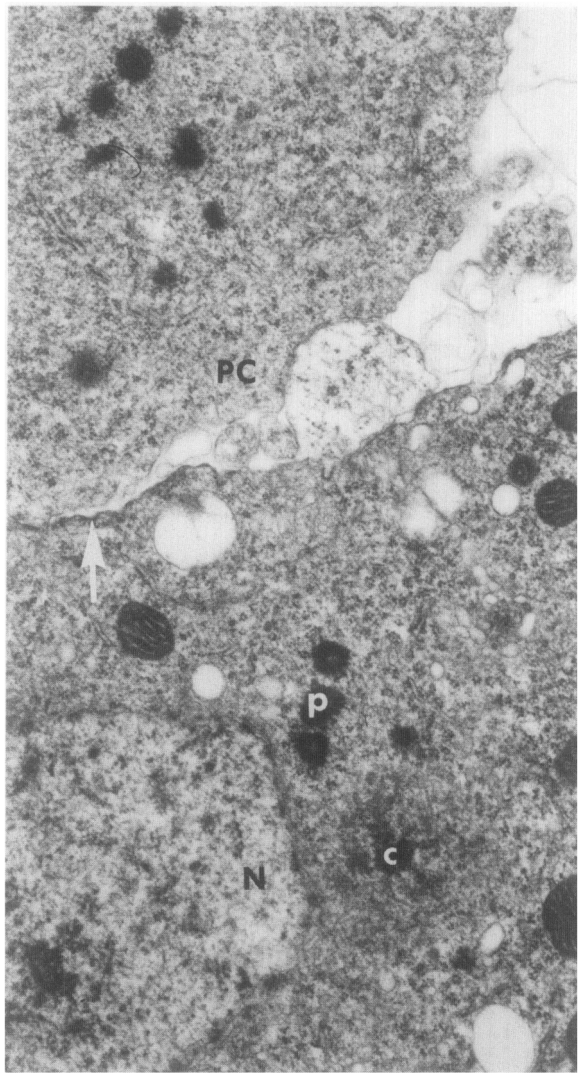

FIG. 7. Electron micrograph of an embryo comparable to Fig. 6A, showing the flattened embryo surface (arrow) below the pole cells (PC). Polar granules (p) are still present around the centriole (c) which is located at the side of the blastema nucleus (N) instead of between the nucleus and the plasma membrane. (×17,000).

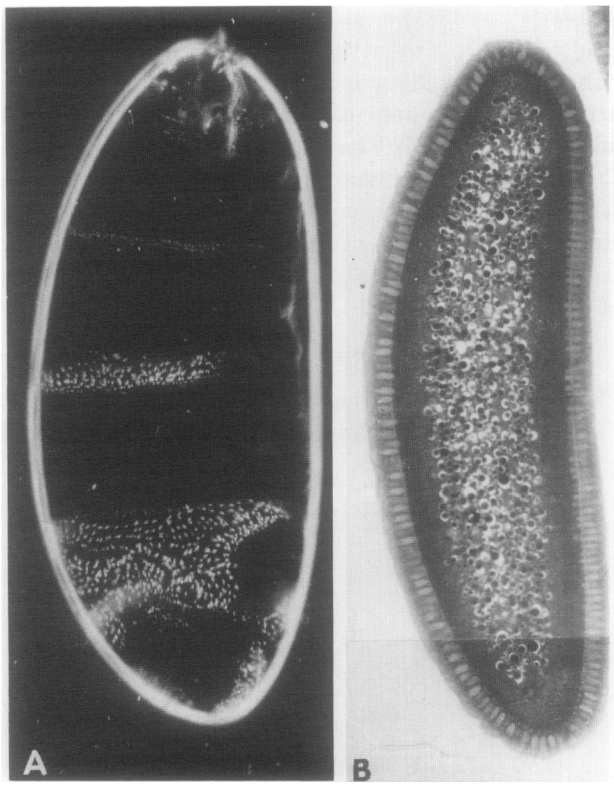

FIG. 8. (A) Cuticle preparation of an embryo from a homozygous *fs(1)N*^{211};*tud*^{3} female, showing the abnormalities of the abdominal segments, characteristic of *tudor*, and the absence of A_8 and analia, due to the *fs(1)N*^{211} mutation. (×60) (B) Histological section of the blastoderm of the *fs(1)N*^{211}/*tud*^{3} embryo, showing the lack of pole cells and concomitant complete cellularization of the peripheral blastoderm layer. (×270)

nate system within the egg to ensure the proper disposition of developmental potential along its axes. Mutations in these genes can disrupt normal development of large sectors of the embryo, as for example in the case of *bicaudal* (Nusslein-Volhard, 1977) in which mirror images of the posterior abdomen are formed. Other genes have been identified whose activities are required during oogenesis for the orderly differentiation of less extensive regions of the mature embryonic pattern (see Konrad *et al.* (1985) for review). In our study we have described in detail a representative of this latter class of mutations. We have shown that the structures which should arise from the terminal 25% of the blastoderm of the *Drosophila* embryo (including parts of the 7th, the 8th to 10th abdominal segments, and all components of the posteriorly derived endodermal components) are missing, except for the production of pole cells. In addition, there are a number of abnormalities at the anterior end of the embryo, which are associated with the anterior endodermal derivatives. Whereas all of the head segments and appendages are visible with the scanning electron microscope between 8 and 12 hr, in some cases there is apparently an inadequate stomodeal invagination leading to the failure of head involution. In other instances, normal head involution occurs, but the pha-

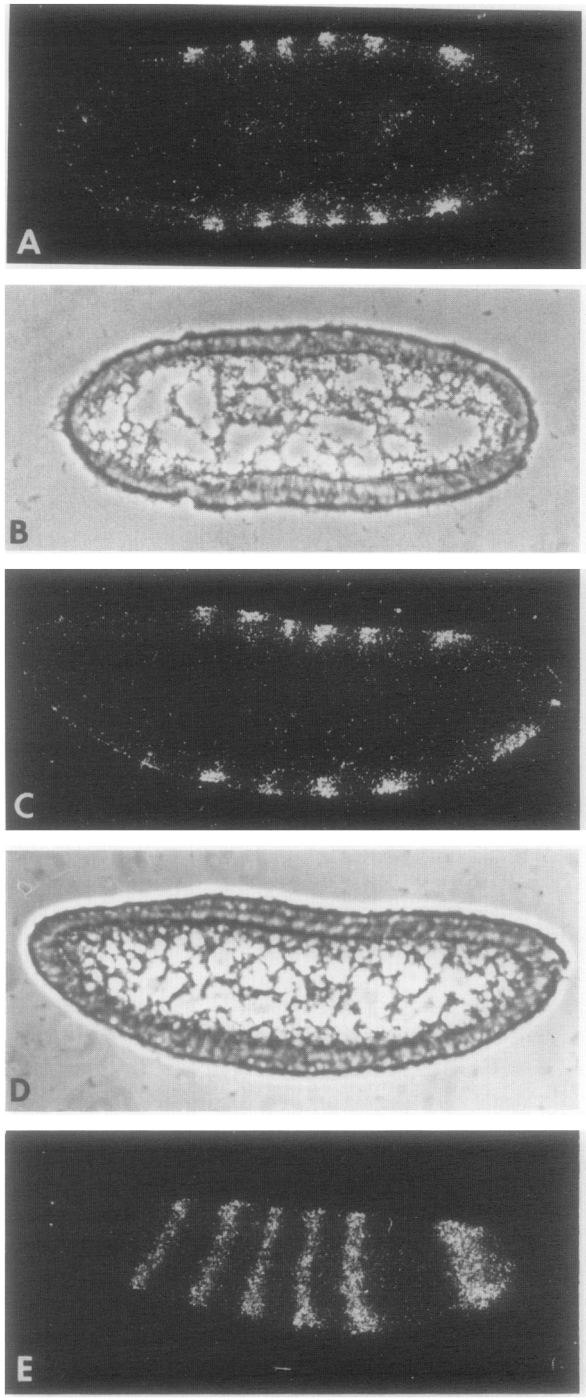

FIG. 9. Autoradiographs (A, C, E) and phase-contrast (B, D) pictures of *fs(1)N*^{211} embryos, hybridized with antisense RNA from the *ftz* gene. At a late blastoderm stage (A, B), only six bands of hybridization are seen. The spacing between bands 5 and 6 is wider than the spacing between the other bands. In a sagittal section of an embryo during early gastrulation (C, D), a larger gap between bands 5 and 6 appears, which can be seen more clearly in a tangential section of the same stage (E). (×120)

ryngeal musculature fails to form and the anterior gut ends either before the proventriculus or immediately after it. Possibly in relationship to the defective anterior gut system, there is extensive cell death in the procephalic neurogenic region, including the stomatogastric ganglia.

Initially, we supposed that the defective cellularization of the posterior blastoderm might be causally related to subsequent developmental abnormalities (cf. Mahowald *et al.*, 1984). However, we have shown that we can obtain a structurally normal posterior blastoderm by eliminating the formation of pole cells through the addition of the *tudor* mutation (Boswell and Mahowald, 1985). In most respects the developmental abnormalities associated with both the *fs(1)N*^{211} and *tudor* mutations are additive in that components of the phenotype of each mutation can be discerned in late embryos. However, defective blastoderm cellularization is completely eliminated. Since we have found aberrant pole cell formation in *fs(1)N*^{211} embryos, we assume that defective blastoderm cell formation is associated with some abnormality in pole cell formation and not causally related to the torso phenotype. It is interesting, however, that this pole-hole effect is characteristic of every torso-like mutation we have examined (cf. Mahowald *et al.*, 1985; Perrimon *et al.*, 1985; Mahowald and Hardy, unpublished results), an observation indicating that the pole-hole and segmentation abnormalities are independent consequences of the same underlying defect.

The origins of the final phenotype are clearly related to changes in the determination of cells at the blastoderm stage. From fate mapping (Hartenstein *et al.*, 1985), genetic marking experiments (Wieschaus and Gehring, 1976; Simcox and Sang, 1982), and *in situ* labeling with DNA probes to segment-specific gene products (Hafen *et al.*, 1984), it is clear that cells at the blastoderm are determined for specific segmental fates. The *ftz*$^{+}$ gene product is expressed in seven evenly spaced bands on the blastoderm in wild-type embryos. Mapping of the labeled zones with respect to segment primordia indicates that the 7th posteriormost zone straddles the 8th and 9th abdominal segments (Hafen *et al.*, 1984; Martinez-Arias and Lawrence, 1985). It is normally positioned at 15% egg length from the posterior tip.

Using the expression of *ftz*$^{+}$-RNA as a molecular indication of the determination state at the blastoderm, we can directly demonstrate that the wild-type fate map is altered in the progeny of *fs(1)N*^{211} females. In contrast to the seven regularly spaced clusters of *ftz*$^{+}$ expression in wild-type, only 6 labeled bands are observed in mutant embryos. The five anterior bands appear to occupy approximately their normal positions. However, the 6th band is markedly set off from the rest and is found in a position close to where the 7th band normally appears. Formally, this could be the result of (i) inhibition of the formation of the normal 6th stripe, implying not only spatial but also functional equivalence of the 6th band in the mutant with the 7th band of the wild-type; or (ii) a posteriorly directed shift in the position of the 6th band, associated with a failure to express the 7th. The cuticle phenotype strongly suggests that the second possibility is realized in the mutant embryo. The 7th expression zone in wild-type marks the position of the 8th and 9th abdominal segments (Hafen *et al.*, 1984), so that the absence of a 7th band in the mutant is consistent with the failure to differentiate abdominal segment 8 and the terminalia in the mutant. According to Martinez-Arias and Lawrence (1985), the 6th *ftz*$^{+}$ expression band represents the primordium of posterior A6 (A6p) and anterior A7 (A7a). In the most extreme cases A7a is the most posterior structure formed in the mutant. We have observed some variability in the relative position and extent of the 6th *ftz*$^{+}$ stripe which is probably reflected in the final extent of abdominal segment 7.

Interestingly, the abnormally large distance between *ftz*$^{+}$ stripes 5 and 6, as well as the increased width of stripe 6, are not reflected in the mutant cuticle phenotype. For instance, if the interband between stripes 5 and 6 represents A5p/A6a (Martinez-Arias and Lawrence, 1985), one might expect an enlargement of A5 and/or A6 in the final cuticle phenotype. This is not the case for the cuticle pattern, although the size of these segments, when first apparent with the scanning electron microscope in 8- to 10-hr embryos, is larger and initially the segment borders are less obvious. It is of interest to note that we have also observed gaps in the embryonic nervous system at the level of A6 following HRP-antibody staining, a result which may also be related to the abnormal expression of *ftz*$^{+}$ between the 5th and 6th stripes. Hence, we suggest that *fs(1)N*$^{+}$ is required during oogenesis for the proper elaboration of the information required to establish the correct developmental fate of the normal posterior region of the embryo. The precise mechanisms by which the *fs(1)N*$^{+}$ gene product affects the transcription and localization of the *fs(1)N*$^{+}$ RNA and the fate of posterior blastoderm cells remain to be clarified. The majority of mutant alleles at the *fs(1)N* locus appear to cause structural damage to the vitelline membrane (Kern, 1979). The notion that the gene might be associated with a plasma membrane funtion in the oocyte could perhaps reconcile the disparate phenotypes observed with different alleles, but further work is required to substantiate this. Because of the dual effect on both anterior and posterior components of the endoderm, it is possible that the primary action of this information is concerned with establishing the potential for endodermal development. The broader effects, especially in the posterior abdomen, may be consequences of this primary disturbance.

The posterior cellularization defect in *fs(1)N*^{211} em-

bryos is intriguing. Normally centrioles are specifically located at the apex of each blastoderm cell and are associated with an array of microtubules (Fullilove and Jacobson, 1971; Karr and Alberts, 1985). However, the defective blastoderm appears to be due both to the abnormal location of the centrosome region and to the loss of its microtubule-organizing center (MTOC) property. It is possible that the excessive pole cell formation, which apparently occurs in fs(1)N^{211} embryos, may deplete the posterior blastoderm of some factor required for maintenance of the centriole as a MTOC. Such a change in centriole function is known to occur at other times during differentiation in *Drosophila.* For example, Mahowald and Strassheim (1970) showed that centrioles became dissociated from nuclei prior to polyploidization of the nurse cells. Similarly, Mahowald *et al.* (1981) found that centrioles in follicle cells, after the last mitotic division, moved from the edge of the nucleus and no microtubules were associated with it. Finally, at the time of the migration of nuclei to the surface of the blastoderm following the 9th synchronous division, the yolk nuclei rapidly become polyploid, indicating the absence of a division center. Thus, we propose that the change at the posterior tip that results in the defective blastoderm may be related to a common developmental process in insects by which cells switch from the mitotic to an endomitotic cycle. In the case of the *fs(1)N^{211}* mutation, the abnormal formation of pole cells leads to an abnormal switch of developmental fate of these posterior blastoderm nuclei to form additional yolk nuclei. It is interesting, in this context, that the ability of the yolk nuclei to cellularize and form a primitive midgut is still present. This may indicate that the mechanism for cellularization of the yolk sac may be by a different mechanism than the process of cellularization at the blastoderm. Further study of the yolk sac is certainly in order.

The developmental roles of the tudor- and torso-like loci appear to be complementary. In the case of tudor-like loci, the germ plasm and the abdominal field anterior to abdominal 8 are affected; in the case of torso-like loci, only structures posterior to abdominal 7 are affected except that pole cells form. Thus, it appears that two sets of maternal effect genes provide the ooplasm information required for the stable establishment of the posterior half of the blastoderm. Future experiments should enable us to decipher the kinds of information provided by these genes, understand the basis for allele-specific phenotypes, and relate these findings to other maternal gene products associated with the major embryonic axes.

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