Cell, Vol. 37, 833–841, July 1984, Copyright © 1984 by MIT

0092-8674/84/070833-09 $02.00/0

Spatial Distribution of Transcripts from the Segmentation Gene *fushi tarazu* during Drosophila Embryonic Development

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Summary

The locus *fushi tarazu* appears to be involved in the establishment of the segmentation pattern of the Drosophila embryo. The cuticle of *ftz* mutant embryos is missing structures in alternating segments such that only half the normal number of segments are present. We have localized *ftz*$^+$ transcripts in tissue sections of wild-type Drosophila embryos by in situ hybridization. Transcripts from the *ftz*$^+$ gene were first detected during nuclear cleavage prior to cell formation. During the last two nuclear divisions *ftz*$^+$ transcription becomes gradually restricted such that at the cellular blastoderm stage the *ftz*$^+$ transcripts are localized in seven evenly spaced bands of cells. The size of each band is similar to the size of the segment primordia at the blastoderm. By the time segmentation becomes morphologically distinct *ftz*$^+$ transcripts are no longer detected. These results suggest that the *ftz*$^+$ gene plays a key role in the determination of the segmentation pattern in the embryo.

Introduction

The generation of a specific number of repetitions of a standard basic unit or segment is a fundamental mechanism underlaying the development of most higher organisms. Little is known about the molecular mechanisms involved in the establishment of spatial organization. In Drosophila, the metameric pattern is most obvious in the segmentation of the external cuticle of the larva and the adult fly. The determination of the segmentation pattern is initiated during the cellularization of the syncytial blastoderm (Chan and Gehring, 1971; Wieschaus and Gehring, 1976; Lawrence and Morata, 1977). The primordia for at least the thoracic and the abdominal segments are formed by a subdivision of the blastoderm in evenly spaced bands of cells, each three to four cells wide (Lohs-Schardin et al., 1979). A cell lineage restriction between neighboring segments occurs at or soon after this stage (Wieschaus and Gehring, 1976).

A number of genetic loci have been identified that appear to be involved in the establishment of the metameric pattern of the Drosophila embryo. Mutations in such genes typically cause embryonic lethality. However, the examination of the cuticular phenotype of the lethal mutant embryos has allowed the assignment of a domain of action for many of these segmentation genes (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard et al., 1982). The isolation and characterization of genes involved in segmentation should provide information on how these genes are involved in the establishment of spatial information.

The locus *fushi tarazu* (*ftz*) is located on the right arm of the third chromosome within the *Antennapedia* gene complex (Wakimoto and Kaufman, 1981; Scott et al., 1983). Embryos homozygous for mutations in this gene die during the terminal stages of embryogenesis. The cuticles of such embryos possess only half the number of segments of wild-type embryos. The ventral denticle belts and adjacent cuticle in alternating segments are missing (Figure 1) (Wakimoto and Kaufman, 1981). On the basis of the mutant phenotype, *ftz* belongs to the class of pair rule mutants as defined by Nüsslein-Volhard and Wieschaus (1980). So far nine loci of this class have been identified, all of which show deletions of different portions of alternating segments (Nüsslein-Volhard et al., 1982).

The phenotype of *ftz* mutant embryos is apparently caused by the absence or inactivity of the *ftz* gene product, since embryos homozygous for deficiencies, which delete the *ftz* gene as well as neighboring genes, exhibit the same deletion pattern (Wakimoto and Kaufman, 1981; Jürgens et al., 1984). If the *ftz*$^+$ gene is expressed autonomously and its gene products are directly involved in the early determinative events that lead to the formation of the segment primordia, it should be transcribed in the progenitor cells of the segmental units that are missing in *ftz* mutants. Furthermore, *ftz*$^+$ expression could then be used to identify those progenitor cells early in development.

To obtain precise information about the spatial distribution and time of appearance of *ftz*$^+$ transcripts during normal Drosophila development we have used in situ hybridization of a cloned genomic DNA segment derived from the *ftz*$^+$ gene to RNA contained in tissue sections of wild-type embryos (Hafen et al., 1983). Here we demonstrate that the *ftz*$^+$ gene is expressed in a segmental manner on the blastoderm and that this segmental pattern of expression is established gradually during nuclear cleavage.

Results

Strategy

To identify and localize cells that contain *ftz*$^+$ transcripts during wild-type Drosophila embryonic development we hybridized the recombinant plasmid p523B to tissue sections of Drosophila embryos. The p523B recombinant contains a 3.4 kb genomic DNA segment that is homologous to most of the RNA coding region of the *ftz*$^+$ gene. Details of the isolation and characterization of the *ftz*$^+$ gene are described in the accompanying paper (Kuroiwa et al., 1984). The p523B recombinant was labeled with tritium by nick translation and afterward directly hybridized to serial frozen tissue sections of wild-type embryos of successive developmental stages. Following hybridization and subsequent removal of the nonspecifically bound

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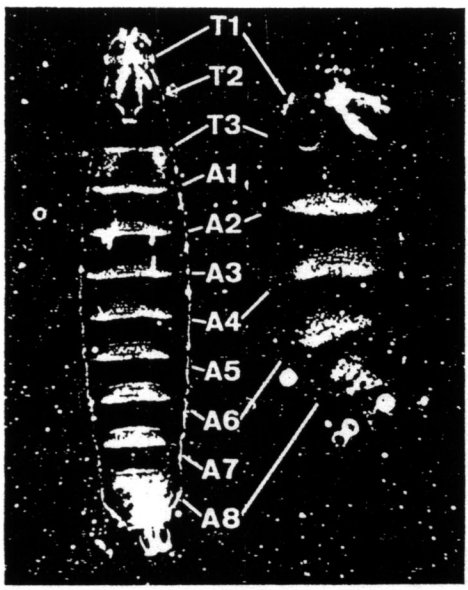


Figure 1. Comparison of the Cuticular Segmentation Pattern of a Wild-Type First Instar Larva and a Lethal *ftz* Mutant Embryo

Cuticular preparations showing the ventral side of a wild-type first instar larva (left) and an embryo homozygous for ftz^{9093} (right) are presented in dark-field illumination. The denticle belts in the anterior portion of each segment are labeled with the corresponding segment number. In the *ftz* mutant only the denticle belts of the uneven-numbered segments are present. The cuticular preparations were obtained as described by Van der Meer (1977), and the *ftz* mutant stock was kindly provided by G. Jürgens. Abbreviations: A1–A8, denticle belts of the eight abdominal segments; T1–T3, denticle belts of the three thoracic segments.

probe the signal detection was achieved by autoradiography. The details of this procedure have been published previously (Hafen et al., 1983). Sections of embryos at the syncytial blastoderm stage were staged by counting the nuclei along the periphery of median sections (Zalokar and Erk, 1976; Foe and Alberts, 1983).

Early Embryogenesis, Formation of the Syncytial Blastoderm

After fertilization, the zygote nucleus undergoes 13 synchronous divisions without the formation of cell membranes. The first seven nuclear divisions take place in the central yolk. After the seventh nuclear division the majority of the nuclei begin to migrate to the periphery, leaving the future yolk nuclei behind. The first nuclei that reach the posterior pole are enclosed by cell membranes after the ninth division to form the pole cells. The remaining nuclei form a syncytium and undergo an additional four divisions. After the 13th nuclear division (2.5 hr after oviposition) the approximately 6500 peripheral nuclei elongate perpendicularly to the egg surface. Approximately 30 min after the nuclei have started to elongate cell membranes begin to grow inward from the surface to enclose the nuclei. After 3.5 hr of development the embryo consists of a single-layer epithelium—the cellular blastoderm (Zalokar and Erk, 1976; Turner and Mahowald, 1976; Foe and Alberts, 1983).

Figure 2a shows a photomicrograph of a sagittal section through an embryo after the ninth nuclear division. The section has been hybridized with the ftz^+ DNA probe p523B and autoradiographed for 21 days. The same section is shown in Figure 2b in dark-field illumination. As a control, parallel sections have been hybridized to the recombinant plasmid p93, which encompasses at least part of a gene that is first expressed at 8 hr of development (McGinnis et al., 1984). Comparison of the grain density obtained with the two probes over embryo sections of the same stage after autoradiographic exposures of equal duration indicates an accumulation of silver grains over sections hybridized to the ftz^+ probe that is 2-fold that over corresponding sections hybridized to p93 (data not shown). It is difficult to assess whether such a small difference in signal intensity is due to the presence of low amounts of ftz^+ transcripts or whether it is merely due to the different probes used. If there are low levels of ftz^+ transcripts present at this stage, these transcripts appear to be evenly distributed in the egg, since no regional difference in grain density can be detected. Similar results were obtained for earlier embryonic stages (data not shown).

Sections of embryos that have completed the 11th nuclear division are shown in Figures 2c–2f. The sections have been hybridized to the ftz^+ probe and autoradiographed for 21 days. Figures 2c and 2d show a horizontal section in bright- and dark-field illumination. A longitudinal section through an embryo at the same stage is shown in Figures 2e and 2f. The peripheral portion in both sections exhibits a higher accumulation of silver grains between approximately 15% and 65% egg length (placing the zero point at the posterior pole). We believe that this observed hybridization signal is due to newly transcribed ftz^+ transcripts, since clusters of silver grains are observed over the nuclei in this region (Figure 2e, arrows and inset). The nuclei close to the anterior pole are unlabeled. This indicates that two nuclear divisions before cells are formed, the ftz^+ gene is already expressed in a spatially restricted manner.

After one further nuclear division, ftz^+ transcripts are still detected between approximately 15% and 65% egg length (Figures 2g and 2h). However, regional differences in the signal intensity are observed within the labeled domain. Comparison of the distribution of silver grains in sections of different embryos at the same developmental stage indicates that the position and extent of the most strongly labeled regions are variable within the labeled domain.

A sagittal section through an embryo that has completed the 13th nuclear division is shown in Figures 2i and 2k in bright- and dark-field illumination. The segmental pattern of the hybridization signal is more obvious at this stage. Three distinct clusters of silver grains can be seen on the ventral side of the section. Each cluster encompasses three to five nuclei, spaced by a stretch of three to five unlabeled nuclei. The signal is less distinct on the dorsal side and in the posterior portion of the section.

Formation of the Cellular Blastoderm

As previously stated, the peripheral nuclei start to elongate after the completion of the 13th nuclear division and cell membranes grow inward from the surface enclosing the nuclei. A sagittal section through an embryo in the process of nuclear elongation and before the completion of cell formation is shown in bright- and dark-field illumination in Figures 3a and 3b. At this stage, the distribution of ftz^+ transcripts shows a regular pattern. Between approximately 15% and 65% egg length seven evenly spaced clusters of silver grains are detected on the ventral and the dorsal sides of the section.

Figure 3c shows a photomicrograph of a sagittal section through an embryo at the cellular blastoderm stage. The corresponding dark-field photomicrograph is shown in Figure 3d. The hybridization signal obtained with the ftz^+ probe is similar to that during nuclear elongation. Seven discrete patches of labeled cells are located between approximately 15% and 65% egg length, evenly spaced by unlabeled cells.

A high magnification of the ventral side of the section shown in Figure 3c is presented in Figure 3e. Each cluster of silver grains encompasses three to five cells and is spaced by three to five unlabeled cells. The number of cells that are labeled in each cluster approximately corresponds to the width of a segment primordium on the blastoderm, which is three to four cells wide (Lohs-Schardin et al., 1979). In a tangential section through the blastoderm epithelium, seven evenly spaced bands of labeled cells are observed (Figure 3f). Further information on the distribution of ftz^+ transcripts along the dorsoventral aspect of the embryos has been obtained by the examination of serial cross sections (data not shown). No significant differences in signal intensities are detected among cells in different positions along the circumference of the embryo. Furthermore, longitudinal sections obtained at various angles always exhibit equally strong labeling on both sides of the section (i.e. Figure 3a). These findings indicate that the ftz^+ gene is expressed in seven discrete belts of cells along the anterior–posterior axis of the blastoderm.

The exact position of the labeled belts of cells with respect to the anterior–posterior embryonic axis is relatively difficult to obtain, since it depends at what angle the sections were obtained. However, the variation of the position of corresponding clusters of labeled cells in similar sections through different embryos was always smaller than 4% egg length, which is the approximate width of a segment primordium (Lohs-Schardin et al., 1979). Thus we conclude that the ftz^+ gene is expressed in the same frame of alternating segmental units on the blastoderm of different embryos.

Gastrulation and Germ-Band Extension

Immediately after the formation of the cellular blastoderm, gastrulation is initiated by the invagination of the mesoderm along the ventral midline. The cephalic furrow is formed on the lateral sides at 67% egg length (Underwood et al., 1980) (Figures 4a and 4c). The cells at the posterior pole, including the pole cells, migrate dorsally and invaginate to form the posterior midgut rudiment. The embryonic germ band is established by the extension of the ventrally located cell layers around the posterior pole on the dorsal side. Thereby, the posterior midgut invagination is moved toward the anterior pole dorsally (for review see Fullilove and Jakobson, 1978).

A horizontal section through an embryo at the beginning of gastrulation is shown in Figure 4a. The corresponding dark-field photomicrograph is shown in Figure 4b. As at the blastoderm stage, the hybridization pattern obtained with the ftz^+ probe consists of seven clusters of labeled cells on either side of the section. The anteriormost clusters are located in the posterior half of the cephalic furrow (arrows).

The fourth cluster of labeled cells on the blastoderm is located in the anterior part of the posterior transverse fold (Turner and Mahowald, 1977) at the early stage of germband extension (Figures 4c and 4d). At this stage, the posteriormost cluster of labeled cells on the blastoderm is located on the dorsal side on both sides of the posterior midgut invagination. Since the pole cells and the underlaying blastoderm cells have moved dorsally and have invaginated, the dorsal and ventral rims of the seventh belt of labeled cells were brought together on the dorsal side.

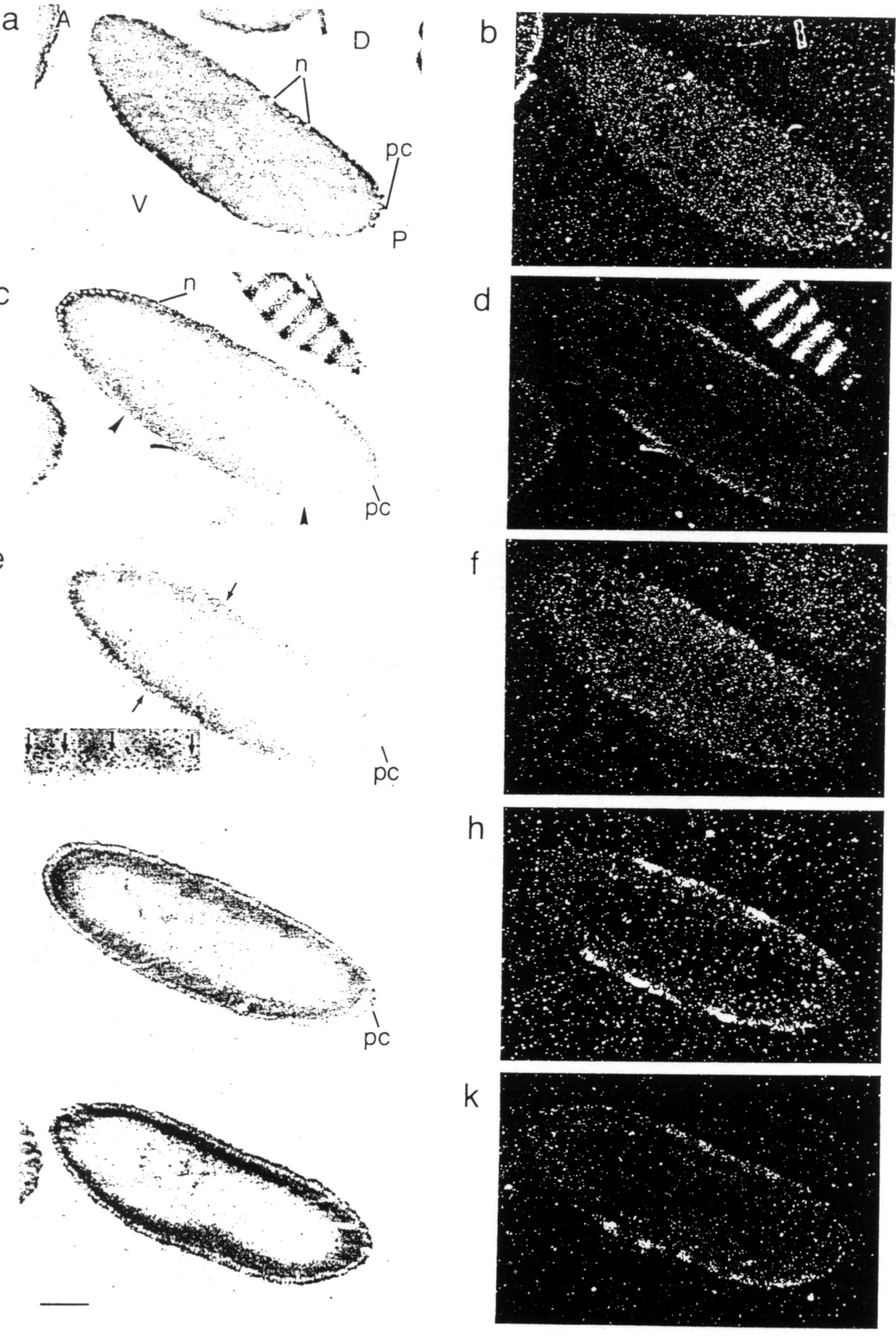
The mesoderm has formed by invagination along the ventral side of the blastoderm and consists of a flat tube of cells inside the ectodermal portion of the germ band. Figure 4e shows an oblique lateral section through an embryo at the same developmental stage as in Figure 4c. A dark-field photomicrograph of the same section is shown in Figure 4f. A portion of the mesoderm is visible in this section. It exhibits the same striped pattern of labeling as the underlaying ectoderm (arrowheads). This hybridization pattern is consistent with the distribution of ftz^+ transcripts in the blastoderm, since ftz^+ transcripts were also detected in the presumptive mesoderm region (Poulson, 1950), located between 10% of the egg circumference on either side of the ventral midline.

Sections of embryos of later developmental stages were also examined for the presence of ftz^+ transcripts. There were no detectable levels of ftz^+ transcripts in embryos older than 4 hr of development. Therefore, it appears that by the time segmentation is first obvious and the germ band is fully extended (4.5 hr of development) the ftz^+ gene is no longer expressed.

Discussion

The principal finding of this analysis is that the ftz^+ gene is expressed in a segmental manner at the blastoderm stage. Each band of labeled cells has approximately the size of a segment primordium, which is three to four cells wide at the blastoderm stage (Lohs-Schardin et al., 1979). Within a band, the labeling extends homogenously in the dorsoventral axis and therefore includes ectodermal and mesodermal progenitor cells.

To determine the developmental fate of cells that contain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ftz$^+$ transcripts, two approaches can be used. First, the position of the labeled cells on the blastoderm can be compared with the fate map for the segmental anlagen at this stage. Second, if the *ftz*$^+$ gene acts cell autonomously, the cells that contain *ftz*$^+$ transcripts should give rise to those segmental portions that fail to develop in the absence of functional *ftz*$^+$ gene product. A detailed fate map of the larval epidermis at the cellular blastoderm has been described (Poulson, 1950; Lohs-Schardin et al., 1979). A comparison of the average positions of the belts of labeled cells and the fat map positions of the thoracic and abdominal segments in percentage of egg length is shown in Figure 5. The anteriormost band of labeled cells, which comes to occupy the posterior part of the cephalic furrow during gastrulation (Figures 4a and 4e), is located anterior to the primordia for the thoracic segments on the blastoderm. When cells were removed from the region of the cephalic furrow, defects in the segmentation pattern of the developing embryo were scored in the posterior head segments (Underwood et al., 1980). Therefore, the cells that are labeled with the *ftz* probe in the posterior part of the cephalic furrow are most likely the progenitors of the posterior head segments. This interpretation is in agreement with the position of the anteriormost labeled band of cells on the blastoderm fate map (Lohs-Schardin et al., 1979), assuming that the posterior head segment primordia are evenly spaced and adjacent to the thoracic primordia.

In *ftz* mutant embryos structures in alternating segments are missing (Wakimoto and Kaufman, 1981; Nüsslein-Volhard et al., 1982; Figure 1). Comparison of the positions of the blastoderm cells that accumulate *ftz*$^+$ transcripts with the fate map positions of the segmental anlagen (Figure 5) strongly suggests that these cells are the progenitors of the segmental portions that are missing in *ftz* mutant embryos. It appears that *ftz*$^+$ expression is required in the progenitors of those structures to ensure their proper development. However, the *ftz*$^+$ gene is not expressed in the intermediate segment primordia, which develop normally in *ftz* mutant embryos. It is possible that there are other genes which exhibit an expression pattern that is out of frame with the *ftz*$^+$ expression pattern. Such genes would be expressed in the intermediate regions where *ftz*$^+$ transcripts are not detected. Good candidates are other members of the pair role class, like the *evenskipped* (*eve*) gene. Embryos homozygous for *eve* exhibit a cuticular pattern that is complementary to the *ftz* pattern (Nüsslein-Volhard and Wieschaus, 1980).

It has been proposed that the segmental anlagen for the embryo at the blastoderm consist of six head segments (Struhl, 1981), three thoracic segments, and ten abdominal segments (Turner and Mahowald, 1977). The domain between 15% and 65% egg length, where the *ftz*$^+$ gene is expressed in alternating segmental units, includes the anlagen for the abdominal, the thoracic, and possibly the last two head segments. However, the *ftz*$^+$ gene is not expressed in the anterior head segments, which might be under a different genetic control. Alternatively, *ftz*$^+$ expression might not be required in the anterior head segments, since the primordia fuse and are extensively modified during development.

The examination of morphological changes of *ftz* mutant embryos during blastoderm formation and gastrulation indicates that the cellular blastoderm is formed normally. Deviations from the wild-type development are first observed when the germ band is extended and segmentation is visible (Nüsslein-Volhard, unpublished results). However, the *ftz*$^+$ gene is only expressed during blastoderm formation and the initial period of germ-band extension, and *ftz*$^+$ transcripts can no longer be detected by the time segmentation becomes morphologically visible. Therefore, it appears that *ftz*$^+$ is a control gene that activates other genes which are required to be expressed later in development to ensure the proper differentiation of the segmental regions missing in *ftz* mutant embryos.

Before proceeding with the analysis of our experimental findings, it is important to indicate a potential problem in the approach we used to determine the site of *ftz*$^+$ expression. The p523B recombinant we used as a hybridization probe to detect *ftz*$^+$ transcripts contains a short DNA sequence that is homologous to at least seven other sequences in the Drosophila genome under conditions of moderate stringency. Some of these sequences are contained in the transcribed regions of the homeotic genes *Antennapedia* (*Antp*) and *Ultrabithorax* (*Ubx*) and probably in other homeotic genes (McGinnis et al., 1984). However, the fact that we do not detect *Antp*$^+$ transcripts in embryo sections as described previously (Levine et al., 1983) when p523B is used as a hybridization probe strongly suggests that our hybridization conditions are sufficiently stringent to detect only *ftz*$^+$ transcripts.

An interesting feature of the *ftz*$^+$ transcription pattern is its establishment during the syncytial blastoderm stage. Two nuclear divisions prior to the formation of cell membranes, *ftz*$^+$ transcripts are detected in the nuclei and in

Figure 2. Distribution of *ftz*$^+$ Transcripts during the Formation of the Syncytial Blastoderm

Tissue sections from 0–3 hr wild-type embryos were hybridized with the *ftz*$^+$ probe p523B, washed, and autoradiographed for 21 days. The anterior end of the sections always points to the left and the posterior end to the right. (a) A sagittal section of an embryo after the completion of the ninth nuclear division; the corresponding dark-field photomicrograph is shown in (b). (c) A horizontal section through an embryo after the 11th nuclear division. The arrowheads indicate that domain, between approximately 15%–65% egg length, that displays a slightly higher hybridization signal. (d) A dark-field photomicrograph of the section shown in (c). (e) A longitudinal section through an embryo at the same stage as shown in (c). The inset is an enlargement of a portion of peripheral domain that exhibits a stronger hybridization signal. The arrows indicate cleavage nuclei that are labeled with the *ftz*$^+$ probe and therefore most likely contain newly synthesized *ftz*$^+$ transcripts. (f) A dark-field photomicrograph of the section shown in (e). (g) A longitudinal section through an embryo after the 12th nuclear division; the corresponding dark-field photomicrograph is shown in (h). (i) A parasagittal section through an embryo after the 13th nuclear division prior to cell membrane formation. The corresponding dark-field photomicrograph is shown in (k). Abbreviations: A, anterior aspect of the embryo; D, dorsal aspect of the embryo; n, peripheral cleavage nuclei, P, posterior aspect of the embryo; pc, pole cells. The horizontal bar indicates a length of 0.1 mm. The embryos were staged on the basis of the number of nuclei per unit area of the cortex, the shape of the nuclei, and the presence of pole cells.

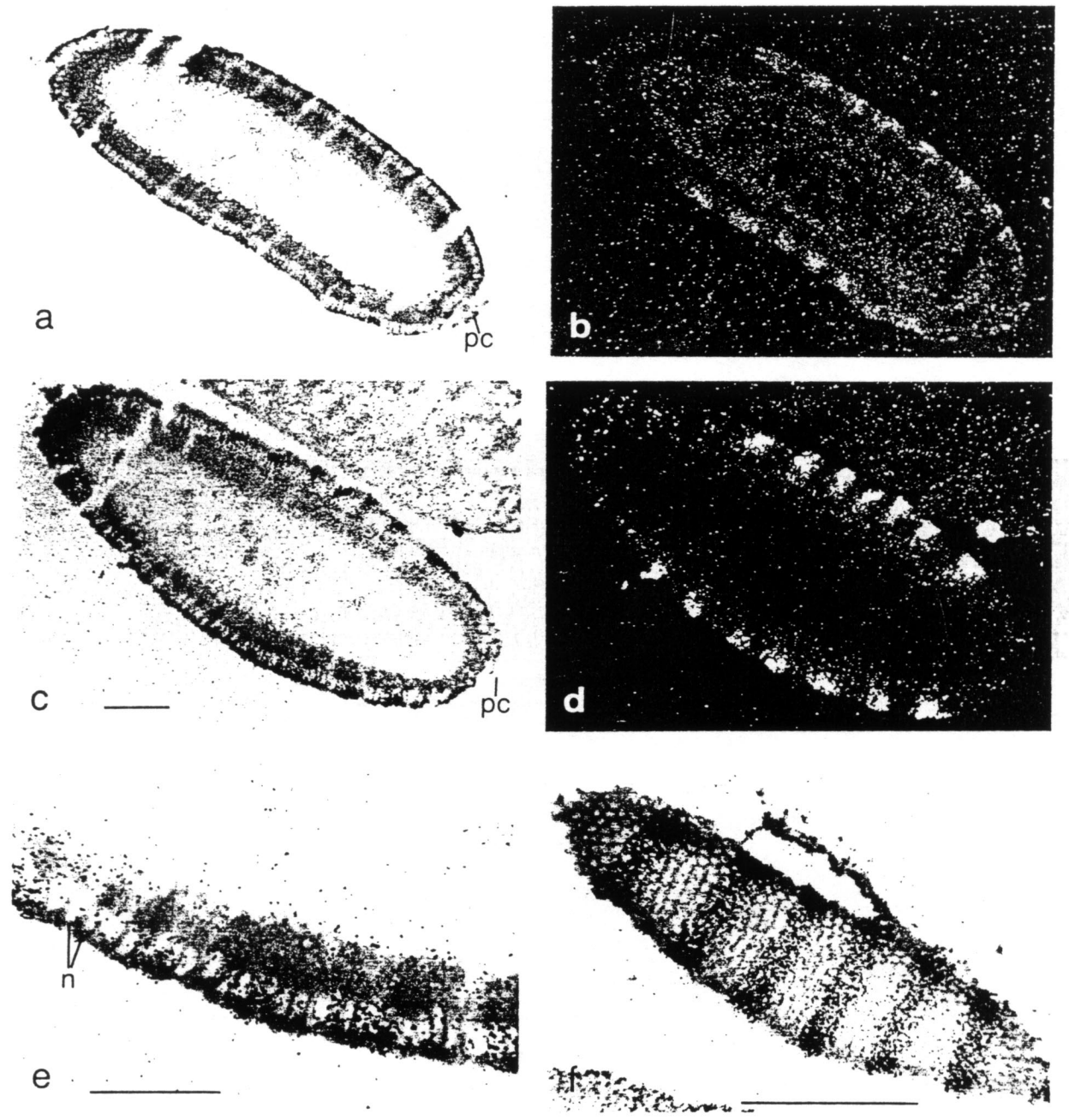

Figure 3. Localization of *ftz*$^+$ Transcripts in Tissue Sections of Embryos during the Cellularization of the Blastoderm

Tissue sections of 3 hr embryos were hybridized to the *ftz*$^+$ probe (p523B), washed, and autoradiographed for 21 days. The orientation of the embryos is the same as in Figure 2. (a) A longitudinal section through an embryo in the process of nuclear elongation and cell membrane formation; the corresponding dark-field photomicrograph is shown in (b). (c) A sagittal section through an embryo of the cellular blastoderm stage (3.5 hr); the corresponding dark-field photomicrograph is shown in (d). (e) An enlargement of the ventral aspect of the section shown in (c). At this stage the nuclei are rectangular in shape and appear white in the preparation. (f) A superficial section through the blastoderm epithelium. The bands of labeled cells correspond to the clusters of labeled cells in the sagittal section shown in (c). Abbreviations are the same as in Figure 1. The horizontal bars represent a length of 0.1 mm.

the peripheral cytoplasm located between 15% and 65% egg length (Figures 2c–2f). Within this domain the transcripts appear evenly distributed. After one further nuclear division, regional differences in the amount of *ftz*$^+$ transcripts are observed within the formerly homogeneously labeled domain (Figures 2g and 2h). Immediately after the last nuclear division the distribution of *ftz*$^+$ transcripts becomes more regular and strong hybridization signals are confined to three to five nuclei, followed by three to five unlabeled nuclei (Figures 2i and 2k). When the nuclei have reached about half of their final length the seven bands of labeling are distinct, although cell membrane formation has

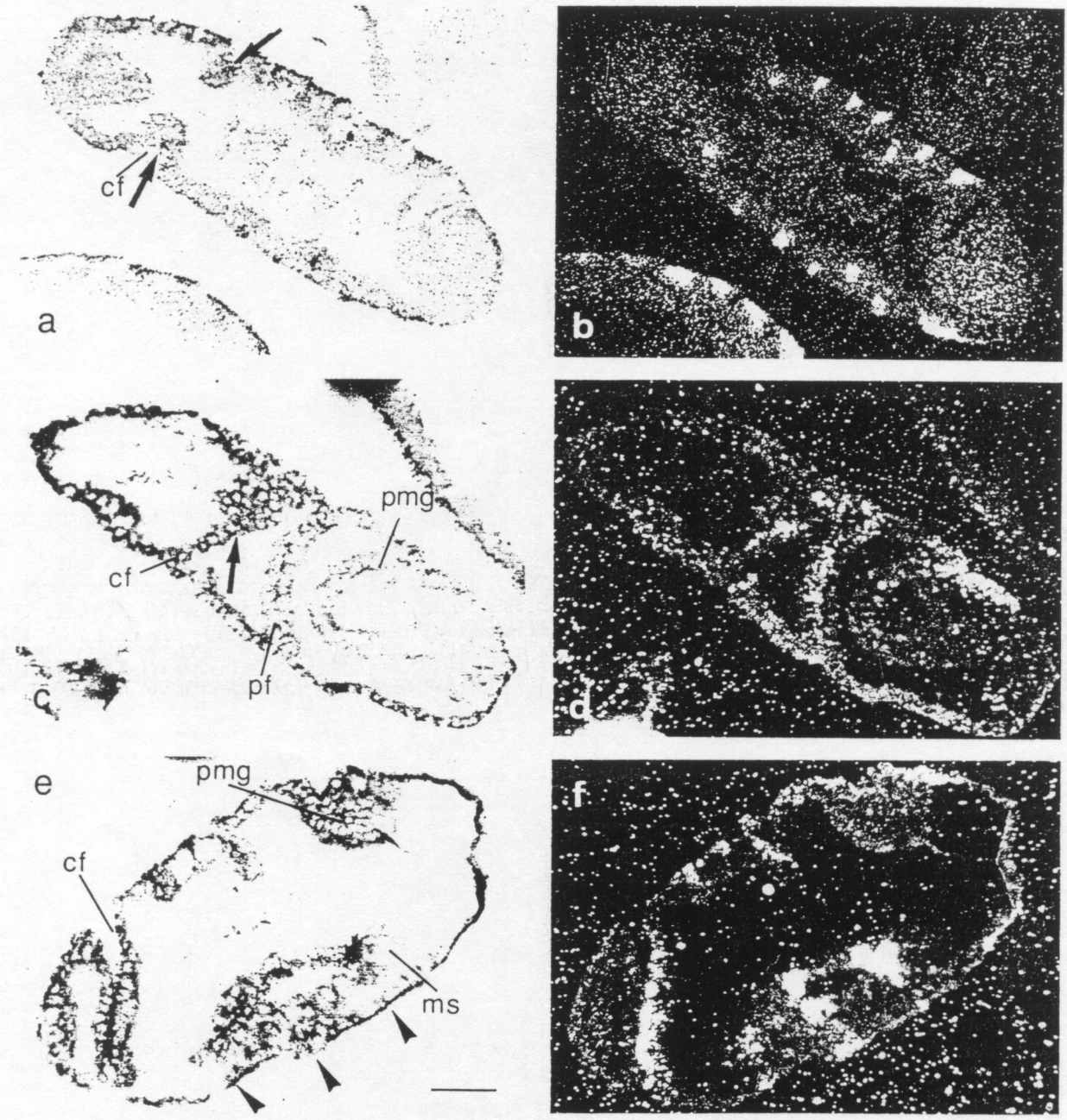

Figure 4. Distribution of *ftz*$^+$ Transcripts during Gastrulation and Germ-Band Extension

(a) A horizontal section through an embryo at the beginning of gastrulation (4 hr). The section was hybridized to the p523B *ftz*$^+$ probe and autoradiographed for 21 days. The arrows indicate that anteriormost cells that contain *ftz*$^+$ transcripts in the posterior part of the cephalic furrow (cf). The corresponding dark-field photomicrograph is shown in (b). (c) A longitudinal section through an embryo in the process of germ-band extension (4 hr); the corresponding dark-field photomicrograph is shown in (d). Autoradiography was for 12 days. The arrows indicate the cells in the posterior part of the cephalic furrow that are labeled with the *ftz*$^+$ probe. (e) An oblique sagittal section through an embryo at a slightly earlier stage than the embryo in (c). A portion of the mesoderm is visible in this section and exhibits the same labeling pattern as the underlaying ectoderm (arrowheads). A dark-field photomicrograph of the section shown in (e) is presented in (f). The section was autoradiographed for 12 days. Abbreviations: cf, cephalic furrow; ms, mesoderm; pf, posterior transverse fold; pmg, posterior midgut invagination. The horizontal bar indicates a length of 0.1 mm.

not begun yet (Figures 3a and 3b). Therefore, it appears that despite the absence of cell boundaries *ftz*$^+$ transcripts remain close to the nuclei in which they were transcribed. This indicates that the peripheral cytoplasm is closely associated with the underlaying nuclei. This observation is consistent with morphological and time lapse studies of developing embryos (Foe and Alberts, 1983), which indicate that each nucleus is associated with a structured domain of cytoplasm containing cytoskeletal elements. The results presented here suggest that the determination

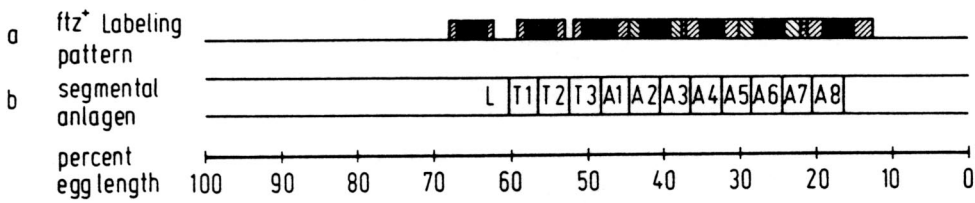

Figure 5. Comparison of the Average Positions of Blastoderm Cells That Contain *ftz*$^+$ Transcripts with the Fate Map Positions of the Segmental Anlagen of Wild-Type Larvae

The positions are given in percentage of egg length, placing the zero point at the posterior pole of the blastoderm. (a) The black boxes indicate the average positions of the seven clusters of cells that contain *ftz*$^+$ transcripts. The hatched areas indicate the variation (standard deviation) of the positions of labeled cells, obtained by comparing different sections. (b) Diagrammatic representation of the positions of the segmental anlagen for the thoracic and abdominal segments with respect to the anterior–posterior embryonic axis according to Lohs-Schardin et al. (1979). Abbreviations: A1–A8, eight abdominal segments; L, labial segment; T1–T3, three thoracic segments.

of the segmental pattern of the embryo is initiated before cells are formed, in part by the position-dependent transcription of the *ftz*$^+$ gene in the nuclei of the syncytial blastoderm. The determined state of the syncytial blastoderm nuclei is reflected in the pattern of *ftz*$^+$ gene expression.

It has been proposed, on the basis of experiments involving the temporal constriction of embryos during nuclear cleavage, that the correct establishment of positional clues that instruct the nuclei and the forming cells in their segmental decisions depends on the interaction of different cytoplasmic regions during nuclear cleavage (Sander, 1976; Schubiger et al., 1977). The gradual restriction in *ftz*$^+$ transcription might be caused by the establishment of positional information during nuclear cleavage.

The nature of the signals or gene product(s) that become localized in the developing embryo to regulate *ftz*$^+$ expression in such a progressive manner is unknown. The analysis of the distribution of *ftz*$^+$ transcripts in embryos that are homozygous for other segmentation mutants might help to identify genes involved in the establishment of positional information. Preliminary results on the distribution of *ftz*$^+$ transcripts in *evenskipped* (*eve*) and *paired* (*prd*) mutant embryos indicates no significant alteration of the wild-type *ftz*$^+$ expression pattern (E. Hafen, unpublished results). Most likely, the best candidates for genes that regulate the *ftz*$^+$ expression are maternal effect or early zygotic genes. Examples of such genes are *bicaudal* (Bull, 1966; Nüsslein-Volhard, 1979) and particularly members of the *gap* mutants (Nüsslein-Volhard and Wieschaus, 1980). These genes appear to control segmentation in a subset of adjacent segments. However, none of these genes appears to control segment formation in the entire domain where the *ftz*$^+$ gene is expressed. Possibly, gene products of different genes regulate the *ftz*$^+$ expression in different regions of the embryos.

Experimental Procedures

A detailed description of the in situ hybridization method used for detecting specific RNAs in tissue sections of embryos has been published previously (Hafen et al., 1983). The hybridization probes used in this analysis had a specific activity of 1×10^8 dpm/μg to 2×10^8 dpm/μg.

Acknowledgments

We are pleased to express our gratitude to Drs. William McGinnis, Michael Levine, Christiane Nüsslein-Volhard, and Gerd Jürgens for constructive discussion and for critical review of the manuscript. We also thank Erika Wenger-Marquardt for excellent technical assistance in preparing the manuscript. This work was supported by a grant of the Swiss National Science Foundation and the Kanton Basel-Stadt.

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Received March 2, 1984; revised April 25, 1984

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